EXOGENOUS MELITURIA IN MAN [1]

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THREE FIGURES

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Frequent studies have been made of meliturias induced in man by the ingestion of sufficient carbohydrate to exceed the assimilation limit. Less consideration has been given to certain exogenous factors of the normal diet. Several investigators (Folin and Berglund, '22; Malmros, '28; Höst, '23; West, Lange and Peterson, '32) have demonstrated a slight melituria, lasting 3 to 4 hours, after mixed meals. Höst ('23) found much of this to be unfermentable and to originate chiefly from breads and other baked or toasted carbohydrate foods. Others have noted additional exogenous sources, including dried fruits, commercial sugar syrups, nuts, chocolate, and apples. In studies of these excretory products which we are reporting, we made use of hydrolysis, bromine oxidation, fermentation and the Sumner/Folin-Wu ratio of glucose equivalents.

METHODS

The analytical, fermentation and bromine oxidation procedures have been previously described (Everett, Shoemaker and Sheppard, '27; Everett, Edwards and Sheppard, '34; Everett and Sheppard, '28; Everett and Edwards, '33;

[1] A preliminary report of these experiments appeared in the biochemical abstracts of the eigthy-fourth meeting of the American Chemical Society, August, 1932.

Everett, '30). Everett, Shoemaker and Sheppard ('27) reported that 0.28 N sulfuric acid was sufficient to give maximal values for hydrolyzable sugar in normal urine, but the buffering effect of urine makes it necessary to use 2.5 N acid in hydrolyzing added lactose, maltose and dextrin. For this purpose we added 1 cc. of 20 N sulfuric acid solution to 7 cc. of urine filtrate in a total sugar tube and heated the mixture 2.5 hours upon the boiling bath. After cooling, 2 cc. of 10 N silicate-free potassium hydroxide solution were added. In control experiments, with sugars added to urine, hydrolysis by the more concentrated acid was an improvement, although not entirely satifactory because of the destruction of some free sugar, especially ketoses. Hydrolysis with N hydrochloric acid produced even greater destruction as estimated by Sumner's method. Deuel, Waddell and Mandel ('26) have reported similar destruction of lower anhydro sugars by hydrochloric acid. Since hydrolysis of urine with a single concentration of acid was obviously deficient in one respect or another, we used both 0.28 and 2.5 N sulfuric acid to secure all possible information. Even with this provision, hydrolyzable sugar determinations have only a semi-quantitative significance.

The experimental subjects were normal adults of both sexes. Night urines were discarded and 1 to 2 hour basal samples collected. The weighed foods were then given, together with 500 cc. portions of water, and urine again collected for the next 3 hours. In most experiments we limited our study to this early excretory period, because the greatest exogenous melituria occurs during this interval (Malmros, '28; Page, '22).

The results, given in figures 1 to 3, are averages of 3 to 10 experiments upon different individuals. Excretions referable to the ingested foods were calculated by subtracting the basal values from those of the corresponding post-prandial periods.

Bread and dextrins

We first investigated the effects of feeding bread. One hundred and fifty gram portions of whole wheat and rye breads caused the excretion of approximately 5 mg. of reducing sugar per hour, together with traces of hydrolyzable sugar. The excreted material could be regarded as dextrins or simpler anhydro sugars, according to one's view of the reactions occurring in baking. It is well known that heating starch results in the formation of dextrins, and that those in the crust are subsequently caramelized. Kalning and Schleimer ('19) believe caramelization to be the principal toasting process. Unfortunately, the words 'dextrinization' and 'caramelization' are used to designate a variety of chemical reactions.

Before attempting further analysis of the excretory products from toast, it is desirable to consider briefly the results of feeding larger amounts of dextrins and anhydro sugars than are possible through the medium of bread. Starch and glycogen may be ingested by man in large quantities without melituria (Allen, '13; Cammidge, '27). Early experimenters believed that dextrins acted similarly, but Folin and Berglund, ('22) found 100 to 200 mg. of hydrolyzable sugar excreted hourly after 200 to 300 gm. of pure commercial dextrin. Unable to confirm this excretion by feeding somewhat smaller amounts of dextrin prepared by amylase, they attributed the excretion to unusable carbohydrate impurities in commercial dextrins. A better explanation is afforded by assuming that they exceeded the assimilation limit of their subject in the first instance, because recent investigators (von Hoesslin and Pringsheim, '23; Lustig and Landau, '32; West, Lange and Peterson, '32) have shown that 100 gm. portions of dextrin cause no melituria—a fact which we have verified by feeding Merck's C. P. dextrin. From these considerations it is obvious that the excretory products from bread and toasted foods are not to be regarded as dextrins.

Maltose is also completely utilized by man (Voit, 1897; Folin and Berglund, '22; Greenwald, Gross and Samet, '24) and

may be dismissed from considerations of exogenous melituria. We have fed 125 gm. of this sugar to normal adults without melituria. In the systemic blood of these subjects the reducing sugar increased 30 mg. per cent by the Folin-Wu method, but the hydrolyzable sugar only 3 mg. per cent, signifying that only traces of maltose arrive at the kidneys.

Toast and anhydro sugars

Nonnenbruch ('25), Kerb and Kerb-Etzdorf ('24) and Grafe and von Schroeder ('24) have shown that man excretes small amounts (usually less than 10 per cent) of ingested lower anhydro sugars. These carbohydrates appear to be absorbed unchanged (Grafe and von Schroeder, '24); cause little increase in blood sugar (Kerb, '24; Peters, '30); and produce no glycosuria in normal or diabetic animals (Deuel, Waddell and Mandel, '26; Grafe, '14; Umber, '15; von Noorden, '17; Reimer, '20). The intermediate anhydro sugars, tetraglucosan and caramel, are partly excreted by dogs, but not by man (Peters, '30; Greenwald, Gross and McGuire, '27). Large amounts of caramel (125 gm.) cause an excretion of considerable unfermentable reducing sugar in man, together with much hydrolyzable sugar (West, Lange and Peterson, '32). After hydrolysis, the latter is fermentable.

According to Grafe ('24), the higher anhydrides, obtained by roasting cereal polysaccharides, are utilized without much excretion, but others (West, Lange and Peterson, '32; Greenwald, Gross and Samet, '24) have found that a highly toasted cereal,[2] causes the excretion of unfermentable sugar (approximately 400 mg. from 100 gm. of the cereal). We have fed 150 gm. portions of this cereal with the results shown in figure 1. There was a marked excretion of reducing material which had a Sumner/Folin-Wu ratio of 1.7. Approximately 70 per cent of this material was non-fermentable, and 60 per cent was not oxidized by bromine in 48 hours. Note that the Sumner/Folin-Wu ratio of the extractable reducing sugar of the cereal is 2.2. One hundred gram portions of ordinary toasted

[2] Grape Nuts.

bread led to the excretion of smaller amounts of reducing material with a ratio near unity (fig. 1). In neither case was hydrolyzable sugar excreted.

These excretory products were not dextrins or their nonfermentable oligosaccharide impurities (Everett and Edwards,

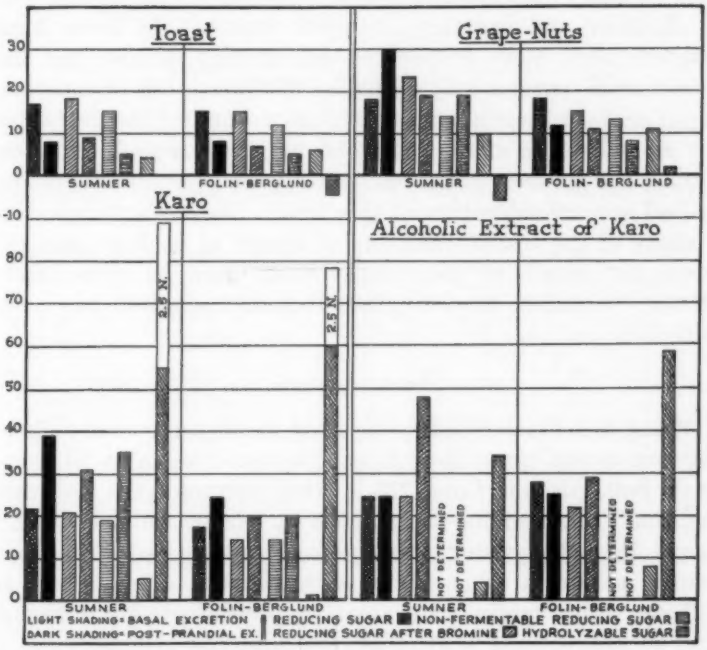

Figure 1

'33) because: hydrolyzable sugar was absent; similar amounts of dextrin caused no melituria; the excretory products were not precipitated by alkaline mercury, while added dextrin was.[3] They resembled lower anhydro sugars in their destruc-

[3] A curious phenomenon results when dextrin is added to normal urine. More reducing sugar appears than can be accounted for by the original oligosaccharide impurities of the added dextrin. The Sumner/Folin-Wu ratio is 2 for the material in urine, but 4.5 for that in the dextrin itself. Previous heating of urine at 100° C. for 5 minutes decreases this effect considerably.

tion by acid, even hydrolysis with 0.28 N acid causing negative hydrolyzable sugar values. In this respect, and also in their greater resistance to bromine oxidation, they differed from caramel. The lack of hydrolyzable sugar differentiated them from ordinary anhydro sugars. The products formed by toasting cereal polysaccharides are not limited to dextrins and ordinary anhydro sugars. At these high temperatures, more deepseated dehydration occurs, and the presence of oxygen may result in the formation of anhydro sugar acids or their lactones. We have searched the literature for an indication of the extent of dehydration and oxidation during the process of starch toasting and have been unable to find any consideration of this topic. We have undertaken a study of the transformations of starch at baking temperatures, but details of these experiments must be given later. It may be stated, however, that lactone formation has been detected.

Glucose syrups

Wang and Felsher ('24) observed melituria in man following commercial granular glucose per os. (See also Blatherwick, Bell, Hill and Long, '25.) They extracted the melituric fraction from commercial glucose by means of alcohol and found that the extract was ineffective when fed alone, but caused melituria when given with glucose. This result is difficult to harmonize with accepted physiologic mechanisms, and we are inclined to attribute the observed phenomena to a difference in behavior of the excreted carbohydrate with the several analytical reagents employed.[4] This glucose impurity should be present in greater concentrations in the commercial syrups from which the granular glucose is crystallized. With this in mind, we made careful studies of the common syrups[5] whose composition is given in table 1.

[4] Dr. C. C. Wang, in a personal communication, informed us that the Folin-Wu method was used for blood sugar and the Benedict-Osterberg method for urine sugar in their experiments.

[5] Karo, blue, red, and orange label.

The results of syrup feeding, as given in figure 1, are averages of many experiments in which we administered 2.7 gm. of blue label syrup per kilogram of body weight. Similar experiments with red and orange label syrups are not detailed here,

TABLE 1

A. Authors' analyses of foods (sugars as per cent glucose)

FOOD	METHOD	REDUCING SUGAR				HYDROLYZABLE SUGAR	
		Original	Non-fermentable	After bromine	S/F-W ratio	0.28 N acid	2.5 N acid
Caramel	Sumner	15	14	7		25	40
	Folin-Wu	12	11	5	1.25	23	37
Dextrin [2]	Sumner	26	22	32		59(78)[1]	70(81)[1]
	Folin-Wu	6	5	7	4.3	67(72)[1]	81(84)[1]
Karo (blue label)	Sumner	47	28			22	20
	Folin-Wu	26	10		1.8	32	34
Karo (alcoholic extract)	Sumner	58				16	
	Folin-Wu	50			1.15	20	
Karo (alcoholic precipitate)	Sumner	36				29	
	Folin-Wu	11			3.3	43	
Hydrol	Sumner	56	17	6		13	21[3]
	Folin-Wu	49	13	5	1.15	19	25[3]
Cane molasses	Sumner	20	4			24	19
	Folin-Wu	19	4		1.05	23	16

[1] Parenthetical values corrected for destruction of oligosaccharide impurities.
[2] Merck's C.P., containing 10 per cent moisture.
[3] Difficultly split by bromine.

From 1000 gm. of blue label Karo obtained 145 gm. of alcoholic extract concentrate and 415 gm. of alcoholic precipitate.

B. Manufacturer's analysis of Karo syrup (per cent)

	BLUE LABEL	RED LABEL	ORANGE LABEL	HYDROL
Moisture	25.3	25.0	25.4	19.5
Dextrins	37.1	35.5	35.6	21.6
Maltose	22.2	21.8	22.5	
Dextrose	7.5	7.3	7.5	56.4
Sucrose	4.8	9.7[1]	9.0	
Invert sugar	2.3			

[1] Calculated from formula.

but yielded almost identical results. The considerable excretion of reducing sugar which followed the ingestion of blue label syrup reached its peak during the second post-prandial hour. In addition there appeared huge amounts of rather difficultly hydrolyzable sugar, which remained unnoticed by Wang and Felsher. This material could be neither dextrin nor dextrin oligosaccharide for reasons already discussed in the preceding section of this report. Fermentation and bromine oxidation indicated that neither maltose nor glucose was excreted.

We prepared an alcoholic extract of the blue label syrup by repeated extraction with 2 volumes of 95 per cent alcohol (table 1). The combined extracts were evaporated to a syrup upon the water bath. Corresponding portions of this syrup (0.4 gms. per kilogram of body weight) and of the alcohol-insoluble fraction (1.1 gms. per kilogram of body weight) were given to individuals who had previously taken the blue label syrup. These experiments indicated clearly that the alcoholic extract (fig. 1) contained practically all of the melituric factor while the precipitate (fig. 2) had none. The hydrolyzable sugar of the alcoholic extract was less than one-fifth of that in the original syrup, yet it was the sole source of the hydrolyzable excretory product. The latter, during the 3 hours under investigation, represented less than 1 per cent of the original hydrolyzable sugar.

These results, differing considerably from those reported by Wang and Felsher, demonstrated that the excretory material from glucose syrups was a reducing saccharide of comparatively low molecular weight. The same oligosaccharide was present in the non-fermentable fraction of hydrol syrup (the commercial designation for the mother liquor from commercial glucose crystals). This non-fermentable fraction has been variously named 'isomaltose', 'gallisin', etc. Berlin ('26) identified 18 per cent of it as gentiobiose. However, the behavior of the excretory product with bromine in our experiments indicated that it was not gentiobiose, but rather an unidentified saccharide of the gallisin

fraction. This excretory product was obviously different from that due to toasted foods. It differed also from the hydrolyzable sugar of normal urine by being split with greater difficulty.

We wish to acknowledge several courtesies of the Corn Products Refining Company and of Dr. Henry Berlin in connection with this part of our investigation.

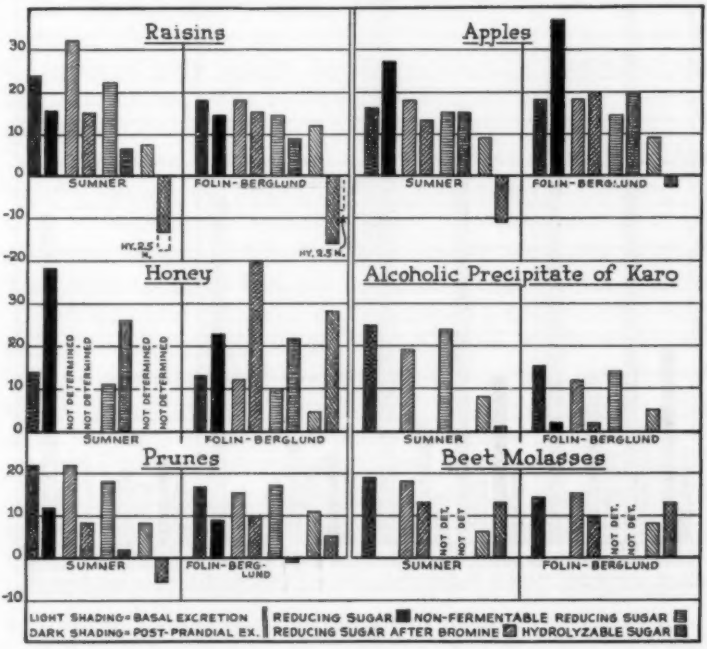

Figure 2

Miscellaneous foods

Experimental findings with other sugar syrups are given in figures 2 and 3. Three grams of honey per kilogram of body weight resulted in the excretion of considerable reducing sugar which was largely non-fermentable and not destroyed

by bromine. In addition, there was an excretion of hydrolyzable sugar which was easily split by bromine (as determined by hydrolysis of the bromine-oxidized filtrates). This material resembled sucrose in several respects, although one would scarcely expect the 4 gm. of sucrose taken by these subjects in the honey to exceed their assimilation limits.

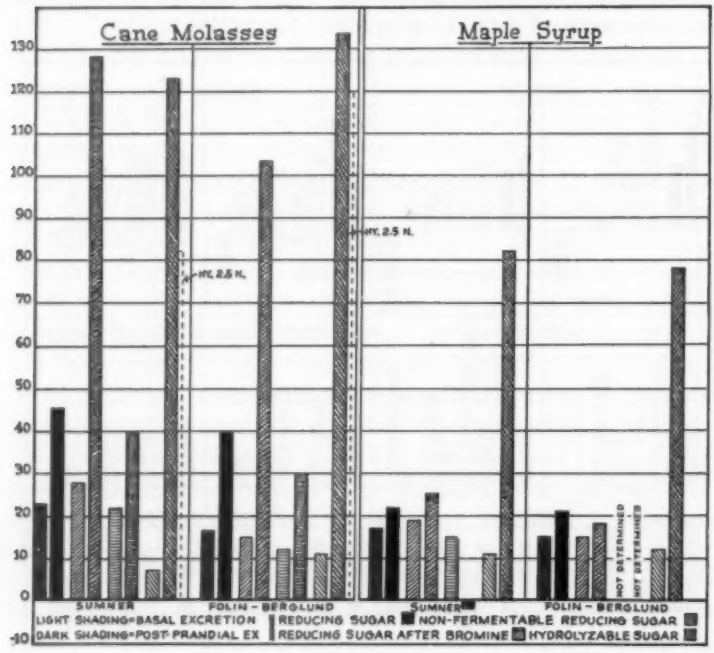

Figure 3

Harding and Selby ('31) found the reducing material excreted after honey to be partly fermentable. West, Lange and Peterson ('32) found it non-fermentable.

Cane molasses, in portions of 2.7 gm. per kilogram of body weight, resulted in the excretion of non-fermentable and bromine-resisting reducing material, together with large amounts of hydrolyzable sugar, which was easily split by 0.28 N acid

and by bromine. Hydrolysis with 2.5 N acid partially destroyed the liberated sugar, which was stable to bromine. The excretory product was evidently a ketose saccharide, but neither sucrose nor raffinose (Everett, Edwards and Sheppard, '34). The molasses fed contained only 50 gm. of sucrose, which should cause no melituria. The fermentable reducing sugar, also noted by Harding and Selby ('31), is a relatively small fraction of the excreted material.

We also fed beet molasses, furnished by the Great Western Refining Company, but only in quantities of 0.35 gm. per kilogram of body weight, because of the high content of salts and extractives. The results were similar to those with cane molasses.

Maple syrup, in portions of 3 gm. per kilogram of body weight, caused the appearance of approximately 20 mg. per hour of fermentable reducing sugar. In one experiment considerable amounts of hydrolyzable sugar were also excreted. This material resembled sucrose.

Five hundred grams of apples caused a considerable excretion of reducing sugar which had a Sumner/Folin-Wu ratio of 0.75. Approximately 50 per cent of this material was fermentable and destroyed by bromine. In other experiments equivalent amounts of cooked apple sauce caused no melituria. It may also be recalled that Folin and Berglund ('22) found a considerable melituria after cider. In our experiments 150 gm. of raisins and 450 gm. of prunes caused similar excretions. With fruits the hydrolyzable sugar of urine tended to be negative, indicating the excretion of rather labile reducing substances. Four hundred grams of bananas and 1000 gm. of peeled oranges caused no melituria, but Harding and Selby ('31) claimed that some fermentable reducing sugar appeared after 500 cc. of orange juice.

Other substances which we found to have no effect were 175 gm. of Fleischmann's yeast, 250 gm. of broiled liver, 500 cc. of cereal beverage and 35 gm. of malt extract.

The hydrolyzable sugar of normal urine

Everett, Edwards and Sheppard ('34) have summarized the properties of normal urine sugars. In considering the hydrolyzable fraction, it may be recalled that parenteral injections of soluble starch (Voit, 1897; Bernard, 1877; von Leube, 1897; Mendel and Mitchell, '05; Verzar, '11), of amylo- and erthrodextrin (Allen, '13; Voit, 1897; Mendel and Mitchell, '05; Mayer, '03), and of glycogen (Allen, '13; Mendel and Mitchell, '05; Mayer, '03; Boehm and Hoffman, 1877; Pavy, 1899) cause the appearance in urine of considerable amounts of hydrolyzable sugar, which has been called achroödextrin. Allen ('13) suggested that the kidney itself was the source of this material, and one might postulate a similar origin for the hydrolyzable sugar of normal urine, which has also been called an achroödextrin (Cammidge, '27). The present investigation has demonstrated that the hydrolyzable sugar of normal urine is easily hydrolyzed and that it is not precipitated by alkaline mercuric reagents. Hence it is not a dextrin. The major portion of this material differs from conjugated glycuronate and also from the hydrolyzable sugar of tungstic acid blood filtrates (Everett and Sheppard, '28). The latter is more difficult to hydrolyze and part of it is precipitated as uranyl nucleotide.[6]

Our metabolic data do not indicate that the carbohydrate foods administered in these experiments stimulated normal sugar excretion. Following toasted foods, syrups and fruits the excreted reducing material resembled the normal uroketose, but the hydrolyzable sugar was either absent or very different; after molasses, the proportion of free to hydrolyzable sugar was very small; the excreted reducing sugar following maple syrup was fermentable, and that from fruits more fermentable than normal sugars; after honey, the Sumner/Folin-Wu ratio for the reducing sugar was high. These metabolites are not identical with the sugar of normal night urine. The reducing uroketose and the hydrolyzable sugar

[6] Unpublished experiments performed in this laboratory by Dr. Wayne M. Hull.

(perhaps two expressions for one substance) remain unexplained metabolic phenomena, whose origin is to be sought in intermediate metabolism. They are not directly traceable to impurities in dietary components and we concur in the opinion of Greenwald, Gross and McGuire ('27) that the excretion of foreign sugars is responsible for only a small fraction of normal urine sugars.

SUMMARY

The authors have investigated the nature of the carbohydrate excretory products of human urine resulting from the ingestion of breads, toasted foods, sugar syrups, fruits, etc. Characteristic metabolites arise from each of these. The substance excreted after toasted cereals is neither a dextrin nor a simple anhydro sugar, but rather an unknown product of starch toasting which is being further investigated. After the ingestion of glucose syrups, there appears an unknown, nonfermentable reducing saccharide of low molecular weight. This material is to be found in the 'isomaltose' fraction of glucose syrups.

Certain phases of the metabolism of dextrins and anhydro sugars have been considered. These substances do not appear to have any relation to the hydrolyzable sugar of normal urine. Methods for determining the latter have been considered.

The origin of the major portion of the uroketose and the hydrolyzable sugar of normal night urine is not to be sought in the articles of diet investigated, but breads and fruits are ordinarily responsible for a small portion of the day sugars.

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VITAMIN STUDIES

XIX. THE ASSIMILATION OF CAROTENE AND VITAMIN A IN THE PRESENCE OF MINERAL OIL

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SEVEN CHARTS

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In a previous paper from this laboratory (Dutcher, Ely and Honeywell, '27), experiments were described which indicated that rats are unable to utilize vitamin A in butter fat when the latter is dissolved in mineral oil. At that time we concluded that "mineral oil may act as a solvent for vitamin A thereby depleting the ingested foods of their supply of this vitamin." Our findings were in general agreement with those of Burrows and Farr ('27) who postulated that unabsorbed mineral oil might act as a solvent for vitamin A in the food and in the mucosa of the digestive tract.

Subsequent experiments in our laboratory indicated quite clearly that the above generalization was not applicable to vitamin A in cod liver oil, since the latter (in mineral oil) seemed to be utilized more efficiently than butter fat when fed at corresponding levels and under similar conditions. These results substantiated the observations of Moness and Christianson ('29), who described experiments in which a concentrate prepared from cod liver oil was dissolved in olive oil and in mineral oil, respectively. These authors concluded

that the utilization of vitamin A from cod liver oil is not appreciably affected by the presence of mineral oil.

Rowntree ('31), working with cod liver oil, concluded that the utilization of vitamin A is lowered by mineral oil only when the cod liver oil is fed at relatively low levels. She expressed the belief that mineral oil can be used with impunity if the vitamin A content of the diet 'is adequate.' Since our interest in the problem relates solely to the question of the use of mineral oil as a vehicle or solvent for vitamin A in the biological assay of food materials, we have purposely confined our vitamin A intakes to those levels which would be considered relatively low.

Jackson ('31) repeated our experiments, using minimal doses of butter fat, and concluded that mineral oil tends to prevent the utilization of vitamin A when butter fat and mineral oil are mixed prior to ingestion, but that practically no detrimental effects can be noted when butter fat and mineral oil are administered separately.

The relatively recent discovery (Moore, '29, '30) that the pigment carotene is the precursor or parent substance of vitamin A seemed to offer a possible explanation for the apparently diverse results obtained with pigmented butter fat and non-pigmented cod liver oil.

The experiments described in the present paper were undertaken to determine the effect of mineral oil on the utilization of, a) purified carotene, b) a pigment-free cod liver oil, and, c) a vitamin A concentrate prepared therefrom, and to ascertain, if possible, the mechanism whereby mineral oil lowers the vitamin potency of butter fat.

EXPERIMENTAL

The rats used in this study were placed on experiment at 21 days of age, at which time they averaged about 40 gm. in weight. All animals were maintained in individual metal cages throughout the entire experimental period. The cages were equipped with false bottoms consisting of heavy wire screening containing two meshes to the inch.

The vitamin A-free ration consisted (in parts per 100) of casein 18, agar 2, dextrin 77, and McCollum and Simmond's no. 185 ('17) salt mixture 3. Vitamins B and G were furnished by daily allotments (0.6 gm.) of yeast which were fed separately from the basal ration to insure a constant and ample supply (Honeywell, Dutcher and Ely, '31) of these vitamins. The basal ration was irradiated with a carbon arc lamp at a distance of 18 inches for 20 minutes (Dutcher and Kruger, '26) to insure sufficient vitamin D for the experimental period.

All animals were depleted of their vitamin A reserves by feeding the basal diet until they showed evidence of vitamin A deficiency, i.e., by the appearance of incipient xerophthalmia or by loss of body weight. At this point the materials to be assayed were fed in measured amounts separate from the basal ration and the curative period was continued for 5 weeks. Evidence for the assimilation of sufficient amounts of carotene or vitamin was considered satisfactory when xerophthalmic symptoms disappeared and when the rate of growth averaged not less than 3 gm. per week during the 5-week curative period. Each experimental group consisted of at least five rats with the exception of a few groups which contained but three or four individuals per group, owing to shortage of animals.

The vitamin A concentrate used in this study was prepared by slight modifications of the method of Drummond, Channon and Coward ('25) which consisted essentially of saponification of 900 gm. of cod liver oil with alcoholic potassium hydroxide, extraction of the unsaponifiable fraction with ether, and evaporation of the ether in an atmosphere of carbon dioxide. Cholesterol was removed from the yellow semi-solid mass by freezing out in methyl alcohol, after which the alcoholic solution of vitamin A was evaporated in an atmosphere of carbon dioxide to a light yellow semi-solid fraction. This fraction, which weighed 0.5 gm., was dissolved in corn oil and made up to a volume of 500 ml. One cubic milliliter of this solution was again dissolved in corn oil and made up to a volume of

250 ml. One-half ml. of this solution was found to contain one Sherman unit of vitamin A, which is equivalent to 500,000 Sherman units per gram of concentrate.

The crystalline carotene used in this study was also dissolved in corn oil and all levels of carotene and vitamin A concentrate were adjusted so that all dosages were administered in ½ ml. of corn oil (daily) in order that variations might be eliminated due to fluctuations in daily intakes of corn oil.

FIRST SERIES OF EXPERIMENTS

In the first series we repeated our former experiments with butter fat, which was fed at two levels, viz., 20 and 40 mg.

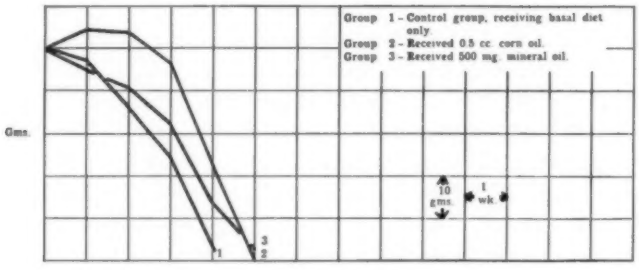

Chart 1

daily. In order to prove that the presence of corn oil had no detrimental effect, two control groups were fed vitamin A-free diets. One of these (group 2) received 0.5 ml. of corn oil (daily) during the curative period. A third control group received the basal diet with additions of 500 mg. of mineral oil (daily) throughout the curative period. The results obtained are summarized in chart 1.

In order to eliminate the possibility of loss of carotene or vitamin A by possible oxidative changes due to the presence of oxidants, two of the butter fat diets (groups 6 and 9) were protected by the anti-oxidant hydroquinone. The results of these experiments are summarized in chart 2.

Groups 10 to 20 inclusive were fed the basal ration supplemented with carotene during the curative period to establish the potency of the carotene employed. It will be noted (chart 3) that 2.5 γ of carotene (daily) were equivalent to one Sherman unit.

Chart 4 summarizes the data obtained when various levels of carotene were fed during the curative period in the presence

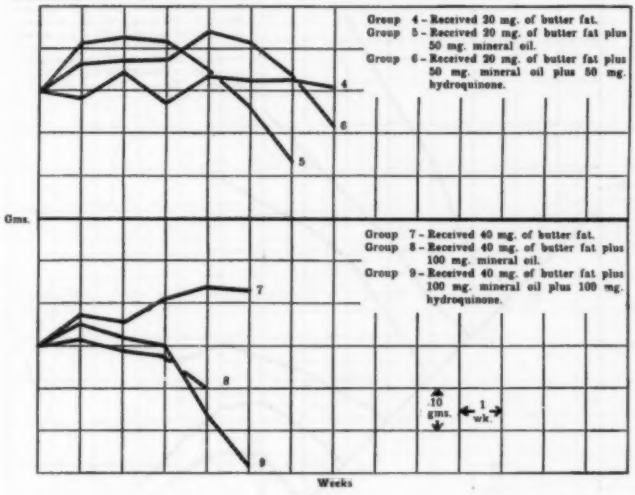
Group 4 - Received 20 mg. of butter fat.
Group 5 - Received 20 mg. of butter fat plus 50 mg. mineral oil.
Group 6 - Received 20 mg. of butter fat plus 50 mg. mineral oil plus 50 mg. hydroquinone.
Gms.
Group 7 - Received 40 mg. of butter fat.
Group 8 - Received 40 mg. of butter fat plus 100 mg. mineral oil.
Group 9 - Received 40 mg. of butter fat plus 100 mg. mineral oil plus 100 mg. hydroquinone.
4
6
5
7
8
9
10 gms.
1 wk.
Weeks

Chart 2

and absence of varying amounts of mineral oil and in the presence and absence of hydroquinone.

Groups 23 to 28 inclusive received varying daily allotments of cod liver oil in the presence and absence of varying amounts of mineral oil. These results are summarized in chart 5.

Chart 6 shows the results obtained when 2×10^{-6} mg. of cod liver oil concentrate were fed in the presence and absence of mineral oil.

Discussion of results obtained in first series of experiments

The results obtained with butter fat dissolved in mineral oil are in general agreement with those previously described

(Dutcher, et al., '27; Jackson, '31). It is clear that mineral oil, in some manner, prevents the utilization of carotene or vitamin A or both. The fact that hydroquinone (chart 2) did not prevent this deleterious effect would seem to support the postulation that the loss of vitamin A activity is not due to the presence of a pro-oxidant in mineral oil.

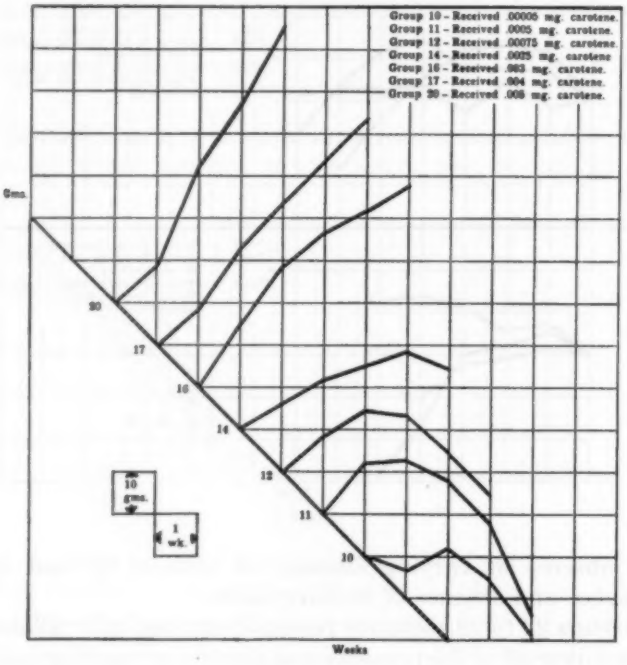

Chart 3

When carotene was fed as the sole source of vitamin A activity, the results were even more convincing (chart 4). When carotene was fed in daily doses of 2.5 γ, as little as 6.25 γ of mineral oil caused marked retardation of growth (groups 14 and 15). When carotene was fed in excess of the Sheman unit requirement (groups 17, 18 and 19), better

growth responses were obtained, but the effect of the small amount of mineral oil was still quite marked. It will be noted, also, that the presence of 100 mg. of hydroquinone did not prevent the deleterious effect of the mineral oil. When the dosage of carotene was increased to 5 γ, growth response was excellent, except where mineral oil was added. When an excessive amount of mineral oil (2 ml.) was fed daily in

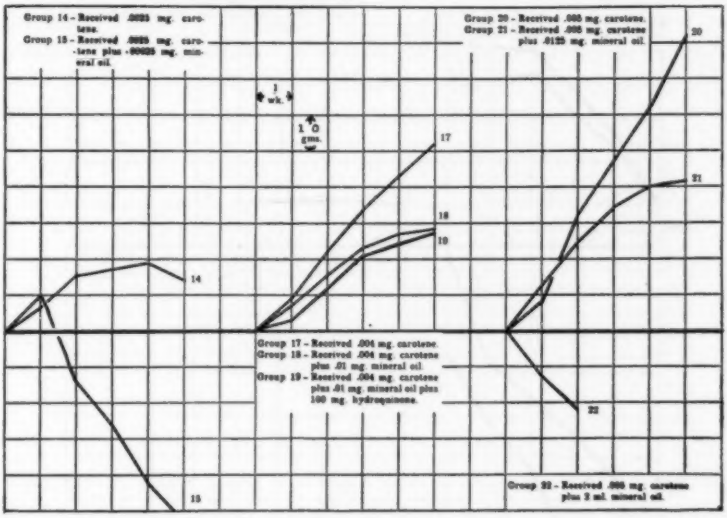
Group 14 - Received .0025 mg. carotene.
Group 15 - Received .0025 mg. carotene plus .00625 mg. mineral oil.
1 wk.
1.0 gms.
14
15
17
18
19
Group 17 - Received .004 mg. carotene.
Group 18 - Received .004 mg. carotene plus .01 mg. mineral oil.
Group 19 - Received .004 mg. carotene plus .01 mg. mineral oil plus 100 mg. hydroquinone.
Group 20 - Received .005 mg. carotene.
Group 21 - Received .005 mg. carotene plus .0125 mg. mineral oil.
20
21
22
Group 22 - Received .005 mg. carotene plus 2 ml. mineral oil.

Chart 4

the presence of 5 γ of carotene (group 22), all animals died within a period of 2 weeks with the usual symptoms of vitamin A deficiency.

When cod liver oil was fed daily (chart 5) at a level of 0.6 mg., which was barely sufficient for maintenance of body weight, there was some evidence that vitamin A utilization was lessened by the presence of 500 mg. of mineral oil. When the dosage of cod liver oil was increased to 1 mg. (daily), which was sufficient for growth, the presence of 500 mg. of mineral oil showed no visible effect on growth response

(groups 25 and 26). Similar results were obtained with groups 27 and 28 when the daily allotments of cod liver oil and mineral oil were increased to 2 mg. and 2 ml., respectively.

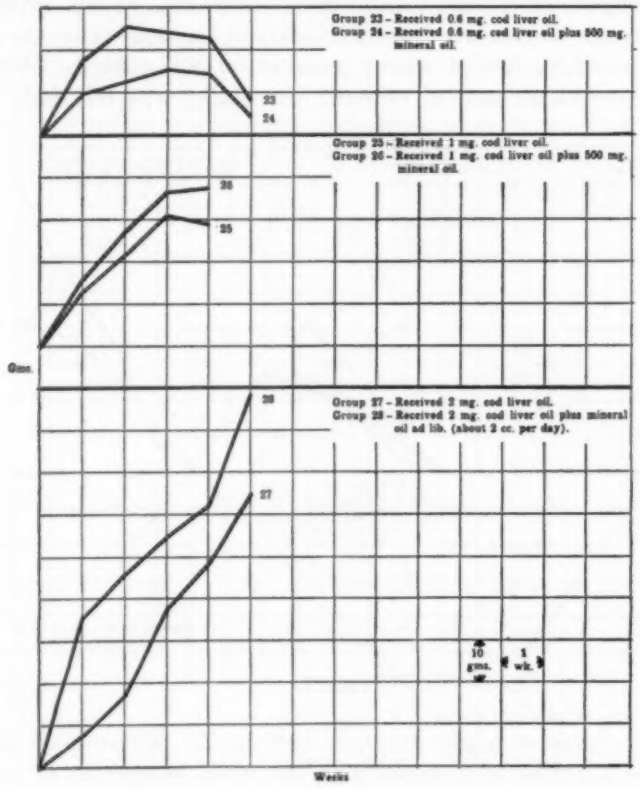
Group 23 – Received 0.6 mg. cod liver oil.
Group 24 – Received 0.6 mg. cod liver oil plus 500 mg. mineral oil.
23
24
Group 25 – Received 1 mg. cod liver oil.
Group 26 – Received 1 mg. cod liver oil plus 500 mg. mineral oil.
26
25
Gms.
Group 27 – Received 2 mg. cod liver oil.
Group 28 – Received 2 mg. cod liver oil plus mineral oil ad lib. (about 2 cc. per day).
28
27
10 gms.
1 wk.
Weeks

Chart 5

When a potent vitamin A concentrate was fed at a level approximating one Sherman unit (chart 6) in the presence and absence of varying amounts of mineral oil, no lowering of the vitamin A utilization could be noted.

It would appear, therefore, that carotene cannot be utilized when ingested in the presence of relatively small amounts of

mineral oil, while vitamin A (per se) is utilized quite efficiently when fed under similar conditions. Since hydroquinone had no tendency to improve utilization in the presence of mineral oil, it would appear that the lack of utilization is not due to oxidative destruction, which was postulated by Olcott and Mattill ('31).

All evidence seems to support the theory that mineral oil possesses a preferential solubility for carotene, preventing its absorption by removing it in solution from the digestive tract. In an endeavor to obtain additional evidence in support of this hypothesis, a second series of experiments was conducted.

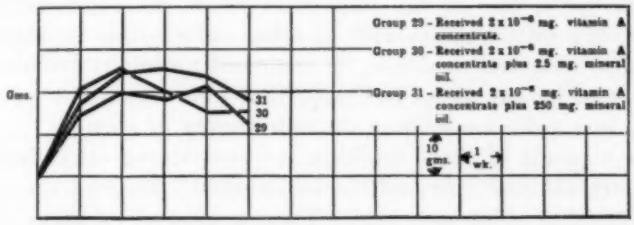
Group 29 - Received 2 x 10⁻⁶ mg. vitamin A concentrate.
Group 30 - Received 2 x 10⁻⁶ mg. vitamin A concentrate plus 2.5 mg. mineral oil.
Group 31 - Received 2 x 10⁻⁶ mg. vitamin A concentrate plus 250 mg. mineral oil.
Gms.
31
30
29
10 gms.
1 wk.

Chart 6

THE SECOND SERIES OF EXPERIMENTS

Prior to this work we had made repeated attempts to separate carotene and vitamin A from feces extracts obtained from rats which received these substances in mineral oil, with the view of re-feeding the carotene or vitamin A thus obtained, to prove, by biological response methods, that these substances are actually excreted in appreciable amounts. None of these attempts yielded satisfactory information owing to our inability to completely remove the mineral oil in order that it might not again vitiate results in the re-feeding period. Some encouraging data were obtained by adsorbing extracts in silica gel and similar adsorbants and by subsequent extraction of the adsorbant with various solvents (Zimmerman and Lachat, unpubl. data). None of these experiments was sufficiently quantitative for our purpose.

We also attempted to solve the problem by feeding varying amounts of carotene and vitamin A concentrate in the presence and absence of mineral oil, using 0.5 ml. of corn oil daily as in series 1. These feeding trials were of the prophylactic type in which the different groups of rats received carotene and vitamin A concentrate from the beginning of the experiment. Feces from these groups were collected daily and preserved under ether and the ether extracts were tested for carotene content with, a) the Lovibond tintometer, b) a colorimeter using a dichromate solution standarized against pure carotene, c) antimony trichloride color measurements, and, d) by spectroscopic measurements. These results were inconclusive, due to the pigmentation of the feces extracts with corn oil pigments and to other interfering substances. We also obtained evidence, by means of antimony trichloride tests and by rat assay methods, that our vitamin A concentrate was quite unstable in the new supply of corn oil.

As a result of these findings, we substituted ethyl laurate for corn oil and repeated the experiments, keeping the ratio of solvent to solute constant. The amounts of carotene, vitamin A concentrate and solvents are summarized in table 1.

Feces were collected daily from groups 32, 33, 34 and 35. These were preserved under petroleum ether during three periods of 10 days each. At the end of each 10-day period the feces were ground in petroleum ether and extracted with this solvent until no more color could be removed. These extracts were evaporated nearly to dryness and were made up to a volume of 10 ml. with redistilled chloroform.

Color values were obtained by comparing the color of the feces extracts with a standard dichromate solution in a colorimeter. No attempt was made to standardize this dichromate solution in terms of carotene, since we were interested primarily in showing whether or not a rough parallelism existed between increased carotene intake and increased carotene excretion.

The relative amounts of yellow pigment excreted during the increasing carotene intakes in the first 10-day period

are indicated graphically in chart 7. Data for the relative amounts of yellow pigment excreted during the second and third feces collection periods are omitted, since they were practically identical with those obtained during the first 10-day period.

TABLE 1

Group 32 Carotene in ethyl laurate	No. of animal	1	2	3	4	5	6
	Amount of carotene	.001 mg.	.002 mg.	.003 mg.	.004 mg.	.005 mg.	.006 mg.
	Amount of solvent	.05 cc.	.10 cc.	.15 cc.	.20 cc.	.25 cc.	.30 cc.
Group 33 Carotene in mineral oil	No. of animal	1	2	3	4	5	6
	Amount of carotene	.001 mg.	.002 mg.	.003 mg.	.004 mg.	.005 mg.	.006 mg.
	Amount of solvent	.05 cc.	.10 cc.	.15 cc.	.20 cc.	.25 cc.	.30 cc.
Group 34 A concentrate in ethyl laurate	No. of animal	1	2	3	4	5	6
	Amount of A concentrate	.01 mg.	.02 mg.	.03 mg.	.04 mg.	.05 mg.	.06 mg.
	Amount of solvent	.05 cc.	.10 cc.	.15 cc.	.20 cc.	.25 cc.	.30 cc.
Group 35 A concentrate in mineral oil	No. of animal	1	2	3	4	5	6
	Amount of A concentrate	.01 mg.	.02 mg.	.03 mg.	.04 mg.	.05 mg.	.06 mg.
	Amount of solvent	.05 cc.	.10 cc.	.15 cc.	.20 cc.	.25 cc.	.30 cc.

In addition to the colorimetric evidence submitted in chart 7, spectrophotometric determinations of carotene were made on two feces extracts [1] using a Konig-Martens spectrophotometer. The samples used were extracts from groups 32 and 33, which received 6 γ of carotene in ethyl laurate and mineral oil, respectively. The values obtained for $-\log_{10}$ of the transmittancy of 2 cm. were 0.41 for the extract from group 32 and 1.49 for group 33, which, according to the graph of

[1] The writers are indebted to Dr. L. J. Briggs, director of the Bureau of Standards, Washington, D. C., for the spectrophotometric determinations of the carotene in these samples.

Schertz ('23), would correspond to 1 and 4 mg. of carotene per liter, respectively.

These values cannot be accepted as quantitative due to the fact that Schertz' measurements were made on carotene dis-

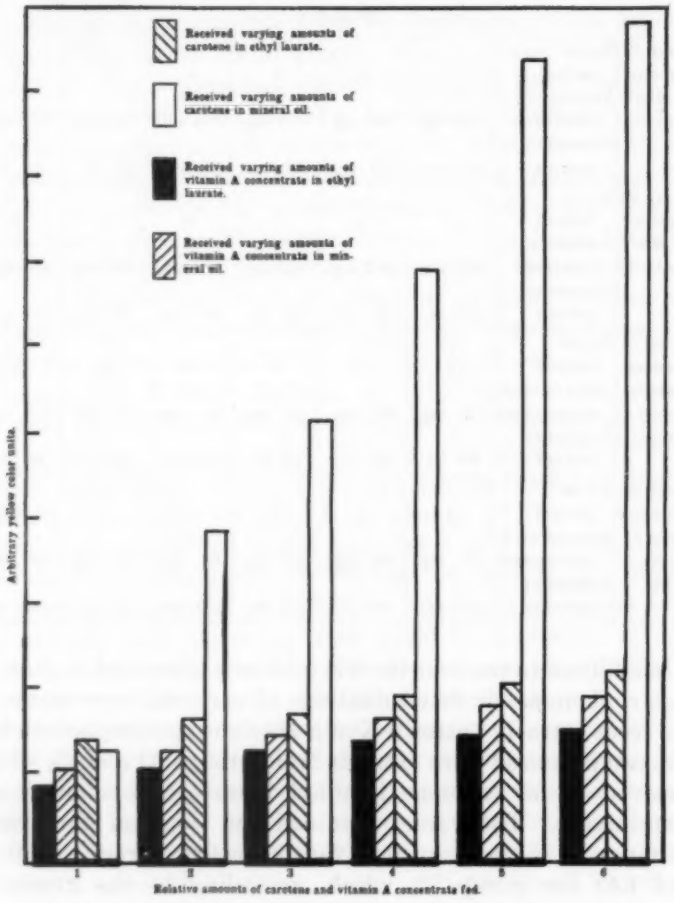
Received varying amounts of carotene in ethyl laurate.
Received varying amounts of carotene in mineral oil.
Received varying amounts of vitamin A concentrate in ethyl laurate.
Received varying amounts of vitamin A concentrate in mineral oil.
Arbitrary yellow color units.
1
2
3
4
5
6
Relative amounts of carotene and vitamin A concentrate fed.

Chart 7 Showing relative intensity of feces extracts when rats received increasing amounts of carotene and vitamin A concentrate in ethyl laurate and mineral oil, respectively.

solved in ether, while our extracts were dissolved in chloroform. It is also certain that our extracts contained interfering substances. Since, however, the feces of both groups of animals were extracted under identical conditions, it would appear that the spectrophotometric data support the conclusion drawn from the color excretion data, viz., that the utilization of carotene is prevented by being excreted from the body in the unassimilated mineral oil.

Discussion of second series of experiments

The question as to the mechanism whereby mineral oil prevents the utilization of carotene is answered in the second series of experiments. Having eliminated, to our own satisfaction, the possibility of oxidative destruction of carotene by a pro-oxidant in mineral oil, we felt that the detrimental effect might be explained by a lack of carotene utilization due to loss from the body by being voided in the unabsorbed mineral oil.

This hypothesis is supported by data obtained when the yellow color was determined in feces from rats which received increasing amounts of carotene in ethyl laurate in the presence and absence of mineral oil. The curve of pigment excretion (group 33) indicates that color excretion was almost in direct proportion to the amount of carotene fed, indicating that the utilization of carotene was almost completely prevented by the mineral oil. Spectrophotometric determinations of carotene in the feces extracts also supported the above conclusion. This was indicated also in the growth response of the rats in groups 32 and 33. We noted also that the growth response in the ethyl laurate groups was somewhat inferior to that obtained in the corn oil groups. This has been observed by other workers.

CONCLUSIONS

1. Our previous results, showing that the vitamin A potency of butter fat is lowered, when fed at low levels in the presence of mineral oil, have been confirmed.

2. When butter fat was fed at higher levels in the presence of relatively small amounts of mineral oil, the deleterious effect of mineral oil was less marked, although the effect could still be noted.

3. The vitamin A potency of cod liver oil and of a cod liver oil concentrate was not adversely affected by the presence of mineral oil, which undoubtedly explains the less harmful effects of mineral oil on butter fat at the higher feeding levels, since a part of the potency of butter fat is due undoubtedly to vitamin A per se, while only a part of the potency can be ascribed to carotene.

4. The harmful effect of mineral oil can be explained on the basis of carotene excretion from the body in the unabsorbed mineral oil. This hypothesis is supported by the fact that yellow pigment excretion (when mineral oil is fed) is roughly proportional to the carotene ingested. This is not true when carotene is fed in the absence of mineral oil. Spectrophotometric determinations of carotene in the feces extracts also support this conclusion.

5. The hypothesis is advanced that the hydrocarbons of the unassimilated mineral oil possess a greater solvent effect on the hydrocarbon carotene than is possessed by the lipids of the intestinal juices, thereby preventing intestinal absorption of carotene. Conversely, it is suggested that the lipids and sterols of the digestive juices possess a preferential solvent effect on the sterol vitamin A, thereby promoting utilization by removing this vitamin from the unassimilated mineral oil.

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THE APPETITE STIMULATING AND GROWTH PROMOTING PROPERTY OF LIVER[1]

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INTRODUCTION

The value of liver in the diet from the standpoint of the supplementary value of its proteins, of its content of mineral elements and water-soluble vitamins B and G, and its therapeutic principles in pernicious anemia has long been recognized. The extent, however, to which liver supplements enhance the nutritive value of a diet which is considered to be complete according to our present knowledge has not received much attention until comparatively recently.

REVIEW OF LITERATURE

The first evidence of a definite acceleration of growth as the result of feeding liver in addition to a complete purified diet was presented by McCay, Dilly and Crowell ('29). By feeding brook trout raw liver in amounts as small as 5 per cent of the diet, a marked acceleration in growth resulted.

Guha ('31), after investigating factors necessary for the normal nutrition of the rat, concluded that the liver from sheep and swine, as well as other dietary articles, contained a growth factor which he was unable to identify with any of the recognized nutrients.

Daggs ('31) concluded from a study of the requirements for lactation of the female dog, that ". . . . liver may contain some hormone or lactation-promoting vitamin . . . ."

[1] Paper No. 1252 journal series, Minnesota Agricultural Experiment Station.

Mapson ('32) has likewise demonstrated the presence of a factor in beef liver that stimulates lactation in the rat and which accelerates growth and improves fertility. He was unable to identify the factor responsible for these beneficial results as the protein, the ash, vitamin B_1, or vitamin B_2 of the liver.

Bahrs ('33) observed an effect upon growth and fertility from the feeding of liver similar to that reported by Mapson. She also found, as did Guha ('31) and Mapson ('32), that the growth response of the males was much greater than that of the females. In none of these reports are data given relative to food consumption or to the effect of the liver upon appetite. It seems highly probable that at least a part of the beneficial results secured from the liver supplement could be explained on the basis of increased food intake as the result of appetite stimulation. This contention receives support from the belief of Palmer and Kennedy ('30) that the growth stimulating effects of fresh lettuce and liver and carrots are due to the effect on food consumption.

Graham and Griffith ('33) observed that when pork liver was fed to rats, there was an increase in growth which was associated with increased food consumption. The authors suggest that the vitamin G in liver may be the cause of its growth stimulus and raise the question as to whether the vitamin G requirements of the rat have been met in the experiments of other investigators who have shown a definite growth stimulus from liver feeding.

It would appear that their own work is open to criticism in that their basal diets were devoid of vitamin G. It would be impossible to demonstrate the presence of a special growth factor in liver by such technic unless the growth rates are compared with growth on similar diets in which the vitamin G is known to be adequate.

That liver may be valuable in the human dietary for reasons other than those commonly given is evidenced by the work of Bartlett ('28) on anorexia in children. His clinical results lead him to suggest that "the liver diet contains some specific

substance which increases an appetite that is depleted and which renews one that is lost."

This cannot, however, be considered as proof that the lack of appetite was originally caused by a deficiency in the diet of a factor needed for appetite stimulation. Such a condition might result from the lack of nutrients needed for growth which could be corrected by the addition of liver.

EXPERIMENTAL

In connection with some rat (Johnson, '33) and swine (Ferrin and McCarty, '30) feeding trials the unusual palatability of liver meal was demonstrated, as well as its ability to make a swine ration palatable that had, heretofore, resisted all attempts to make it so, regardless of the combinations of supplements used. The inclusion of two parts of this product in the swine ration, in place of the two parts of tankage used in the protein supplemental mixture of the controls, increased feed consumption by approximately 27 per cent. In the search for another material that had similar properties, a beef muscle product was obtained which had been processed according to the same procedure as the liver meal. An experiment with rats (Johnson, '33), comparing the two products when mixed into diets to the extent of approximately 5 per cent, showed that both products had similar appetizing qualities.

In order to obtain some information as to the effect of these supplements, as well as of fresh liver, upon appetite and growth, the following procedure was instituted, using the rat as the experimental animal.

The basal diet used consisted of the following ingredients: dextrin, 65.5 per cent; purified casein, 20 per cent; lard, 10 per cent; and Hawk and Oser's ('31) salt mixture, 4.5 per cent. In addition, 500 mg. of yeast tablets that had been immersed in cod liver oil previous to feeding were given to each rat daily.

The groups and supplements fed are shown in table 1.

Ten rats, five males and five females, were used in each of groups 1 to 4. The basal diet was fed ad libitum. Group 5a consisted of four males and five females which were paired with four males and five females in group 5b. They were fed according to Mitchell and Beadles' ('30) paired-feeding method. The control rat of each pair received an additional amount of the basal diet equivalent to the dry matter contained in the 0.5 gm. fresh liver given to its pair mate. Each rat was started on experiment at 28 days, kept in an individual cage, and fed its basal diet in a feed pan and its additional supplements in a small dish. Pork liver was selected for this study because it was available at all times at the Animal Husbandry Meat Laboratory and because it was desired to determine whether or not pork liver would have an effect upon growth similar to beef liver, especially in view of the fact that in the majority of investigations beef liver had been fed. In groups 2 and 3, 0.15 gm. of the liver meal and beef muscle meal were fed because it was desired to feed the same weight of dry matter as was supplied by the 0.5 gm. of fresh liver.

Results with pork liver, liver meal and beef muscle meal

The average growth and food consumption per week of the rats in each group are shown in table 2.

A consideration of the data in table 2 reveals that the fresh liver supplement increased both food consumption and growth as compared to group 1, which received only the basal diet. The liver meal likewise had a favorable effect on growth and food intake, but not to the same extent as did the fresh liver. During the experiment it was interesting to note that while the liver meal was consumed readily the beef muscle meal was apparently relished more than was the liver product, as evidenced by the time required for the complete consumption of the two products. Since the beef muscle product was liked even better than the liver meal, but since it did not result in an improvement of growth, it would seem that liver

contains some factor that stimulates growth either directly through a stimulation of the growth processes or indirectly through a stimulation of appetite. The former assumption seems to be more plausible, for it would seem that the avidity

TABLE 1

Supplement additions to basal diet

GROUP	ADDITIONAL DAILY SUPPLEMENTS
1	none
2	0.5 gm. fresh pork liver
3	0.15 gm. liver meal
4	0.15 gm. beef muscle meal
5 a	none
5 b	0.5 gm. fresh pork liver

TABLE 2

Food[1] consumption and growth data of rats in a study of the effect of pork liver, liver meal and meat meal upon appetite and growth

WEEK	GROUP 1		2		3		4		5		
	Basal		Basal + 0.5 gm. fresh pork liver		Basal + 0.15 gm. liver meal		Basal + 0.15 gm. beef muscle meal		a Basal	b Basal + 0.5 gm. fresh pork liver	
	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1st	24.6	44.1	27.8	44.9	25.8	44.8	25.4	44.5	19.6	39.9	19.3
2nd	17.6	48.4	24.5	51.1	21.9	47.1	19.2	47.7	19.1	50.5	21.2
3rd	20.5	53.1	28.3	59.2	24.0	57.6	21.8	54.5	22.4	52.9	21.2
4th	22.5	53.2	26.3	63.0	25.1	60.1	22.9	52.8	20.2	48.8	20.2
5th	13.7	57.2	19.6	65.9	17.3	61.1	15.4	55.0	14.4	52.6	15.5
Total	98.9	256.0	126.5	284.1	114.1	270.7	104.7	254.5	95.7	244.7	97.4
Average for 5 weeks	19.78	51.20	25.30	56.82	22.82	54.14	20.94	50.90	19.14	48.94	19.48

[1] The data for food consumption for groups 5a and 5b include the additional food given to 5a to correct for the dry matter in the liver supplement given to 5b.

with which a material is eaten would be an indication of its ability to stimulate the appetite. The results of group 5 throw some light upon this question. The gains between the pair mates are equal for 1 week and, for the remaining 4 weeks,

favor the controls for 2 weeks and the liver fed rats the other 2 weeks. This fact and the fact that the differences are of small magnitude forces one to the conclusion that the liver did not act as a growth stimulant when food consumption was controlled and that, therefore, in groups 2 and 3 the beneficial results from liver feeding must have been due to appetite stimulation even though the liver was not mixed as part of the regular diet. The statistical analysis of the data of the paired experiment, given in table 3, shows that there is a probability of 0.87858, i.e., odds of approximately 7 to 1, that the mean difference in total gains between pair mates was due to the liver supplement.

TABLE 3

Statistical[1] results of paired-feeding experiments with rats fed purified diet and purified diet plus 0.5 gm. of fresh liver daily

MEAN DIFFERENCE BETWEEN PAIR MATES IN TOTAL GAIN IN WEIGHT, GRAMS	STANDARD DEVIATION	Z VALUE	PROBABILITY
+ 1.66	3.684	0.452	0.87858

[1] 'Student' ('08).

After the above experiments had been completed, a report by Seegers and Smith ('32) of an attempt to determine in what manner liver exerts its favorable influence upon growth came to the writer's attention. By the use of the paired-feeding method they found that rats receiving the liver supplement grew at a faster rate than the controls.

An inspection of their data reveals that while equal amounts of the basal diet were given to both groups there was apparently no adjustment made for the amount of nutrients given in the liver supplement. During the 40-day experimental period this would amount to approximately 20 gm. of dry material, and when taken into consideration greatly minimizes the significance of the difference in growth between the controls and those receiving the liver.

For future studies of this appetizing factor it was considered desirable to determine the relative content of it in

livers from different species as well as in livers from young animals as compared to older ones. Pork, beef and calf livers were selected for this study. In order to obtain samples of liver representative of these two species, portions of ten pork livers and ten beef livers were secured from a local packing plant. Because of the expense, only one calf liver was obtained. Each kind of liver was first frozen and then ground and thoroughly mixed. The mixtures from which the daily supplements were taken were kept frozen.

The groups and the supplements they received are given in table 4.

TABLE 4

Supplement additions to basal diet

GROUP	ADDITIONAL DAILY SUPPLEMENT
1	none
2	0.5 gm. fresh pork liver
3	0.5 gm. fresh beef liver
4	0.5 gm. fresh calf liver
5 a	none
5 b	0.5 gm. fresh beef liver

Ten rats, five males and five females, were used in each group. The basal diet was fed ad libitum to groups 1 to 4. Group 5a consisted of five male and five female rats which were paired with five males and five females in group 5b. These were fed according to Mitchell and Beadles' ('30) paired-feeding method. The same procedure was followed in this experiment as has been described in the first experiment.

Results with pork liver, beef liver and calf liver

The average gain in weight and food consumption per week of the rats in each group are shown in table 5.

The data for the first four groups emphasize the desirable effect of the liver supplements upon growth and food consumption. The three kinds of liver apparently vary but little in their content of the factor responsible for these results. That the factor had no effect whatever upon growth when food intake was controlled is shown by groups 5a and 5b.

It is interesting to note that the male rats showed a greater response to the liver supplement than did the females. This is shown in table 6, which gives the averages of the controls and of those receiving the fresh liver in both experiments. The difference in growth of the males on the two diets, however, is due entirely to greater food consumption, for when food intake was controlled, as in the paired experiments,

TABLE 5

Food[1] consumption and growth data of rats in a study of the effect of pork liver, beef liver and veal liver upon appetite and growth

WEEK	GROUP 1		2		3		4		5		
	Basal		Basal + 0.5 gm. fresh pork liver		Basal + 0.5 gm. fresh beef liver		Basal + 0.5 gm. fresh veal liver		a Basal	b Basal + 0.5 gm. fresh beef liver	
	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain	Food	Gain
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1st	25.0	54.7	35.1	62.4	34.0	61.0	32.7	58.0	19.9	46.8	20.9
2nd	24.6	64.4	32.7	73.8	38.3	77.0	34.8	73.2	23.8	56.1	24.4
3rd	24.7	69.4	32.8	85.0	32.3	87.3	33.1	86.6	28.0	67.4	28.0
4th	20.4	69.6	27.2	88.2	30.0	91.9	28.5	85.3	22.2	66.9	19.9
5th	16.7	74.3	20.7	88.4	23.3	94.7	22.2	89.2	17.0	66.0	17.1
Total	111.4	332.4	148.5	397.8	157.9	411.9	151.3	392.3	110.9	303.2	110.3
Average for 5 weeks	22.28	66.48	29.70	79.56	31.58	82.38	30.26	78.46	22.18	60.64	22.06

[1] The data for food consumption for groups 5a and 5b include the additional food given to 5a to correct for the dry matter in the liver supplement given to 5b.

TABLE 6

The average weekly gain in weight and food consumption of male and female rats[1] in the study of the effect of liver upon appetite and growth

DIET	SEX			
	Males		Females	
	Gain	Food	Gain	Food
	gm.	*gm.*	*gm.*	*gm.*
Basal + Liver	33.73	79.10	20.17	68.83
Basal	22.67	60.90	18.91	56.26

[1] Basal + Liver, 21 males and 19 females.
Basal, 11 males and 9 females.

growth of those receiving the liver was no greater than that of the controls.

SUMMARY AND CONCLUSIONS

The effect of several meat supplements upon growth and appetite was studied with rats. In the first experiment fresh pork liver, when fed as a 0.5 gm. daily supplement to a purified diet, increased growth and food consumption as compared to the controls receiving only the basal diet. When food consumption was controlled by use of the paired-feeding method, the rats receiving the liver supplement did not grow significantly faster than the controls. Liver meal, when fed in amounts equivalent to the fresh liver, increased the growth rates of the rats receiving it to a lesser degree than the fresh liver. Beef muscle meal, although more appetizing than the liver meal, when fed as a supplement to the basal diet did not increase food consumption. This result, in view of the similar appetizing properties of the two products when mixed as parts of the diet, is evidence that the palatability of a food itself is not a measure of its ability to stimulate the appetite for other foods with which it is not mixed.

In the second experiment the appetizing properties of fresh pork liver, beef liver and calf liver and the effect upon growth of fresh beef liver when food intake was controlled, were studied. The three kinds of liver were found to increase food consumption and growth to a similar extent. The increased growth, however, is entirely the result of appetite stimulation as shown by the equal growth of controls and those receiving the beef liver when food intake was equalized.

Male rats show a decidedly greater growth response to fresh liver supplements than do females. This is due in part to a greater consumption of food by the males and also to the fact that they utilized the greater food intake much more efficiently than the females.

These experiments confirm the conclusion of Palmer and Kennedy ('30) and suggest a different interpretation of the growth resulting than from a so-called 'growth' factor. The factor should be referred to solely as an appetite factor.

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THE UTILIZATION OF ENERGY PRODUCING NUTRIMENT AND PROTEIN AS AFFECTED BY INDIVIDUAL NUTRIENT DEFICIENCIES

II. THE EFFECTS OF VITAMIN B DEFICIENCY [1]

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ONE FIGURE

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INTRODUCTION

It is evident, in the light of the current literature, that vitamin B must serve important functions in connection with the utilization of nutriment—functions which reveal themselves through the profound disturbance of metabolism when the supply of this vitamin is inadequate.

Especially in relation to the intermediary metabolism of carbohydrate, and therefore to energy metabolism, does it appear that vitamin B plays a prominent role. Also, the pathological changes resulting from vitamin B deficiency, even if not directly related to the metabolism of energy and protein, may indirectly affect the disposition of such nutrients in various ways.

Graham and Griffith ('33) studied the effect of vitamins B and G on the utilization of food for growth, and based conclusions on the average caloric requirement per gram of gain in weight, by young rats. Their results, such as are comparable on the basis of equal and fairly adequate caloric intake, indicate an increased consumption of food calories per gram

[1] Publication authorized by the director of the Pennsylvania Agricultural Experiment Station, February 13, 1934, as technical paper no. 635.

of total body gain, in case of rations depleted of both vitamins B and G. The evidence is less conclusive with regard to rations low in either vitamin B or G alone.

Evidence exists in the growth data recently presented by Sure, Kik, Walker and Smith ('33), of an effect of vitamin B deficiency on the utilization of food energy. On the same food intake rats on deficient and supplemented diets differed as much as 50 gm. in weight as a result of a growth period of about 15 weeks. Mitchell ('33) also presented data from which he concluded that rats which received a vitamin B supplement grew faster than did their pair-mates, whose food differed only in that it contained less of this factor. A question is raised as to the significance of these findings, however, by growth curves presented by Kon ('29), indicating that the average growth of groups of three rats each, fed a vitamin B deficient diet, is generally similar to the average growth of groups of rats fed an equal quantity of a complete diet; also by experiments of Hogan and Pilcher ('33), using systems of controlled and ad libitum feeding, which seem to show that the growth of rats deprived of the vitamin B complex does not differ significantly from others consuming equal quantities of a complete diet; and further by the results of the experiment to be discussed.

Conclusions as to effects of dietary deficiencies, based on growth data alone, are often compromised as a result of differences in food intake, and are also rendered uncertain because of lack of evidence as to the composition of the body increase. Also, in many experiments involving vitamin B deficiency, difficulty arises from the fact that it is impossible to distinguish effects due primarily to loss of appetite from others resulting from the nutritive defect itself.

The primary purpose of the experiment to be discussed was to observe the effect of vitamin B deficiency on the utilization of energy and protein, but secondary effects are also reported. The experimental subject was the albino rat; the paired method of food control was employed; and conclusions were based on metabolism, growth and body analysis experiments.

EXPERIMENTAL PROCEDURE

The details of technic were as in the first of this series of papers (Swift, Kahlenberg, Voris and Forbes, '34), twelve pairs of rats being fed, for a period of 13 weeks, on a synthetic ration of the following composition: vitamin-free casein 18 per cent, Osborne and Mendel salt mixture 4 per cent, crisco 10 per cent, dextrin 64 per cent, cod liver oil 2 per cent, cellu flour 2 per cent. This ration contained 93.5 per cent dry matter, 2.51 per cent nitrogen, and 4632 small calories per gram. Autoclaved brewer's yeast was fed separately, as a source of vitamin G and other heat-stable yeast factors. It analyzed 94.0 per cent dry matter, 8.21 per cent nitrogen and 4267 small calories per gram. Vitamin B was fed in the form of a special concentrate prepared and donated by Parke, Davis & Co. The potency of this concentrate, in terms of the Sherman unit was given, by Parke, Davis & Co., as one unit per milligram.

The basal ration was fed in equal quantities to each rat of a pair, the rat consuming the smaller quantity of food determining the intake of its pair mate. No refused food was discarded, the food intake of the last day of each weekly period being so restricted as to equalize the intake for the week, and so that no food remained for that day. Thus the weekly intake was kept the same for each rat of a pair.

The autoclaved yeast and vitamin B concentrate were weighed separately in quantities sufficient for a weekly allotment. The approximate daily allowance was then taken from these measures and added to each day's food. The intake of autoclaved yeast, which was kept the same for the two rats of each pair, varied from 0.2 gm. per day at the start to 0.5 gm. per day after about the fourth week. It represented about 10 to 15 per cent of the total food consumed, a quantity considered adequate for normal nutrition.

The rat of each pair which received the supplemented diet was given 3 to 4 Sherman units of the B-factor daily, while its pair mate received an occasional unit per day, as required to maintain slight but continuous growth. Aside from this

difference in vitamin B content the two diets were identical in composition.

Additional assurance of the fact that the basal diet was deficient in vitamin B is contained in the record of food refusals. Among all the pairs of rats the individuals on the deficient diet averaged 48.9 refusals for the entire experiment, as compared with an average of 7.3 refusals for the rats on the supplemented diet. The loss of appetite by the individual on the deficient diet became apparent by the end of the second week of feeding.

The general care and management of the rats, as well as the method followed in making the daily collections of urine and feces and in the preparation of these samples for analyses, has been previously described (Swift, Kahlenberg, Voris and Forbes, '34). At the end of 13 weeks, by which time most of the rats had at least doubled in weight, the rats were killed and analyzed in the manner reported in the foregoing reference.

The rectal temperature of each rat was taken on 3 consecutive days of each week during the last 3 to 5 weeks of the experiment. This was done at about 10 o'clock in the morning, before the day's food was given, and after water had been withheld for about 2 hours.

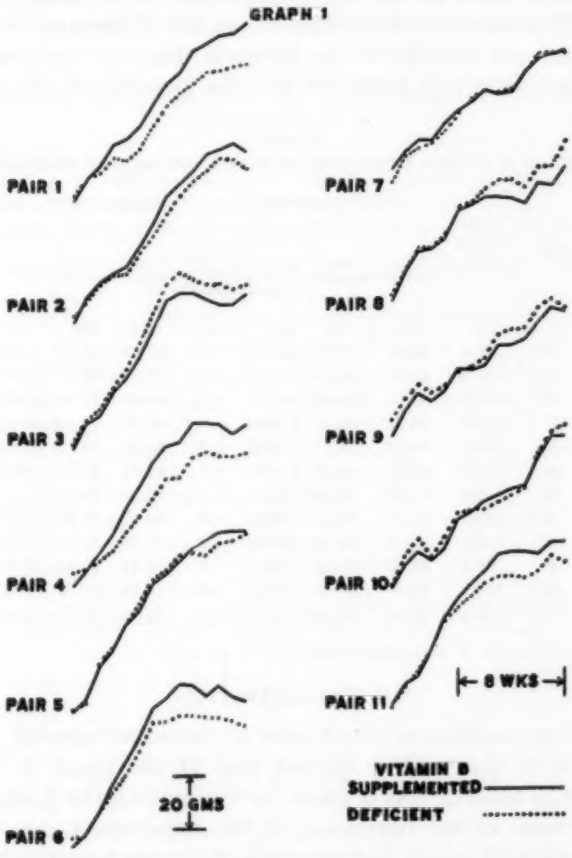
In reporting the data one pair of rats is omitted, because of an error in determining the sex.

RESULTS OF EXPERIMENT

Growth

Under the system of controlled food intake followed, it is possible to attribute differences in rate of growth of pair mates to the vitamin B deficiency alone. The growth curves appear in figure 1, and a comparison of the quantities of growth is contained in table 1. These data fail to reveal any consistent restriction of the rate of growth which may be attributed to a lack of vitamin B. The certainty of this conclusion, as it is related to the data in question, may be substantiated by statistical analysis. Among a possible 143

comparisons of weekly gains, the rat on the supplemented diet exceeded its pair mate (deficient diet) seventy-three times; the rat on the deficient diet exceeded its mate sixty-five times; and in five comparisons both rats gained the same.



An outcome such as this, among a series of events which may happen with equal probability in either of two ways, deviates but 4 from the ideal expectancy and is less than the calculated standard deviation.

In another view of the same data—the difference in weight gained or lost, per week, between the individuals of each pair of rats—it is found that the average of the 143 differences is $+0.327 \pm 0.114$ gm., the plus sign indicating advantage in favor of the rats which received the supplemented diet. The odds are only 18 to 1 that this difference is significant and are insufficient to indicate that the supplemented diet was definitely superior for the production of gain in weight.

TABLE 1

Effect of vitamin B deficiency on growth and on food utilization

PAIR NO.	SEX	FOOD INTAKE (DRY MATTER)	VITAMIN B DEFICIENT DIET				VITAMIN B SUPPLEMENTED DIET			
			Initial weight[1]	Final weight[1]	Gain	Dry matter of food for 1 gm. gain	Initial weight[1]	Final weight[1]	Gain	Dry matter of food for 1 gm. gain
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	F	383.9	48.46	97.65	49.19	7.8	46.83	113.88	67.05	5.7
2	F	347.4	43.62	98.10	54.48	6.4	42.08	105.74	63.66	5.5
3	M	367.5	45.30	103.44	58.14	6.3	43.61	99.90	56.29	6.5
4	F	343.8	46.83	89.23	42.40	8.1	42.13	98.54	56.41	6.1
5	M	380.3	45.34	108.24	62.90	6.0	44.50	110.34	65.84	5.8
6	M	337.3	46.97	88.47	41.50	8.1	44.07	96.76	52.69	6.4
7	F	343.3	43.69	90.86	47.17	7.3	48.32	89.39	41.07	8.4
8	M	348.9	41.43	99.12	57.69	6.0	39.43	90.57	51.15	6.8
9	M	327.0	53.49	96.19	42.70	7.7	47.49	92.43	44.94	7.3
10	F	339.6	42.06	99.50	57.44	5.9	41.14	93.81	52.67	6.4
11	M	363.1	45.70	98.58	52.88	6.9	46.91	106.20	59.29	6.1
Aver.		352.9	45.72	97.22	51.50	6.9	44.23	99.78	55.55	6.4

[1] Minus contents of alimentary tract.

BODY COMPOSITION

The composition of the bodies of the experimental rats, at the end of the feeding period, and of the gains in weight, appear in table 2. As a basis for estimating the composition of the rats at the beginning of the experiment, ten rats of the same age, which had received the same treatment as the subjects of the experiment, were killed and analyzed. The average results, expressed in relation to the empty fresh weight, are as follows: ether extract, 7.30 per cent; total nitrogen, 2.77 per cent; gross energy, 1.65 Cal. per gram.

The average per cent of alimentary 'fill' for these ten rats was 9.15 per cent, and this figure was used in estimating the initial empty weight of the experimental rats.

From the data of table 2 it is evident that the composition of body gains made was remarkably variable, and that growth data by themselves are not critically significant as measures

TABLE 2

Effect of vitamin B deficiency on the ether extract, nitrogen, and energy contents of the bodies of rats

PAIR NO.	COMPOSITION OF BODIES OF RATS[1]			COMPOSITION OF BODY GAINS			TOTAL GAINS		
	Ether extract	Nitrogen	Energy	Ether extract	Nitrogen	Energy	Ether extract	Nitrogen	Energy
	Vitamin B deficient diet								
	Per cent	*Per cent*	*Cals. per gram*	*Per cent*	*Per cent*	*Cals. per gram*	*Gm.*	*Gm.*	*Cals.*
1	6.34	3.44	1.78	5.39	4.11	1.91	2.65	2.02	93.9
2	9.57	3.32	2.05	11.40	3.76	2.37	6.21	2.05	129.4
3	4.46	3.47	1.63	2.24	4.02	1.62	1.30	2.34	94.2
4	7.40	3.37	1.87	7.50	4.03	2.11	3.18	1.71	89.5
5	6.17	3.44	1.75	5.36	3.91	1.83	3.37	2.46	114.8
6	3.49	3.48	1.53	−0.82	4.29	1.40	−0.34	1.78	58.1
7	6.39	3.47	1.83	5.53	4.11	1.99	2.61	1.94	93.8
8	8.06	3.39	1.91	8.62	3.83	2.10	4.97	2.21	120.9
9	4.75	3.47	1.68	1.57	4.36	1.71	0.67	1.86	73.0
10	8.67	3.17	1.94	9.68	3.45	2.15	5.56	1.98	123.5
11	4.26	3.57	1.61	1.63	4.25	1.58	0.86	2.25	83.7
Aver.	6.32	3.42	1.78	5.28	4.01	1.89	2.82	2.05	97.7
	Vitamin B supplemented diet								
1	7.26	3.45	1.85	7.23	3.92	2.00	4.85	2.63	133.9
2	10.46	3.24	2.11	12.55	3.55	2.41	7.99	2.26	153.3
3	5.91	3.44	1.76	4.83	3.96	1.85	2.72	2.23	104.3
4	10.39	3.33	2.16	12.69	3.74	2.53	7.16	2.11	143.0
5	6.61	3.43	1.83	6.14	3.89	1.95	4.04	2.56	128.2
6	3.88	3.47	1.57	1.01	4.06	1.51	0.53	2.14	79.4
7	11.37	3.26	2.27	16.14	3.82	3.00	6.63	1.57	123.3
8	5.00	3.48	1.64	3.23	4.03	1.64	1.65	2.06	83.8
9	6.90	3.32	1.81	6.48	3.89	1.97	2.91	1.75	88.7
10	11.40	3.22	2.20	14.62	3.57	2.63	7.70	1.88	138.5
11	6.26	3.53	1.80	5.45	4.13	1.92	3.23	2.45	114.0
Aver.	7.77	3.38	1.91	8.22	3.87	2.13	4.49	2.15	117.3

[1] Minus contents of alimentary tract.

of nutritive values of diets. Thus, while it has been shown that a deficiency of vitamin B in all probability did not affect the rate of growth of the rats in this experiment, the data giving the composition of the bodies, and of the tissues produced during growth, do reflect significant effects of the deficient diet.

As a result of the low vitamin B intake the bodies of the rats which had received the deficient diet contained less fat and energy than did their pair mates. The ether extract averaged 6.32 per cent, and the gross energy 1.78 Cals. per gram, in the bodies of the rats which had received the deficient diet, as compared to 7.77 per cent ether extract and 1.90 Cals. per gram, in the bodies of the rats on the supplemented diet. The difference is more strikingly reflected in the calculated fat and gross-energy contents of the gains produced. The average ether extract in the gains of the deficient rats was 5.28 per cent, or 2.82 gm., while in the rats which received the supplemented diet the corresponding values were 8.22 per cent or 4.49 gm. The average energy in the gains of the deficient rats was 1.89 Cals. per gram, or 97.7 Cals gained; and in the rats which received the supplemented diet, 2.13 Cals. per gram, or 117.3 Cals. gained. Thus, the deficient rats made 37 per cent less gain in ether extract, and 17 per cent less gain in body energy than did the rats which received the supplemented diet.

The percentage of nitrogen in the body gains averaged 4.01 for the rats on the deficient diet and 3.87 for the rats on the supplemented diet, but this difference is entirely accounted for by the larger percentage of fat in the bodies of the latter group.

UTILIZATION OF ENERGY

The digestibility of energy-producing nutriment (table 3) was the same for both diets.

The metabolizable energy, as reported, is not corrected for the non-metabolizable energy of the protein stored. For the purpose of this experiment, the interpretation of which is based largely on comparative data, the metabolizable energy

refers to the energy intake minus the total energy lost in urine and feces. Energy losses in the urine were consistently larger for the deficient rats, the odds being about 800 to 1

TABLE 3

Effect of vitamin B deficiency on the digestibility, and the metabolizable energy of the food, and on the loss of heat from the body

PAIR NO.	ENERGY INTAKE	ENERGY OF FECES	ENERGY OF URINE	DIGESTIBLE ENERGY		METABOLIZABLE ENERGY		HEAT LOSS	
				Total	Per cent of intake	Total	Per cent of intake	Total	Per cent of intake
Vitamin B deficient diet									
	Cals.	*Cals.*	*Cals.*	*Cals.*		*Cals.*		*Cals.*	
1	1886.6	131.7	79.7	1754.9	93.0	1675.2	88.8	1581.3	83.8
2	1705.9	117.5	66.0	1588.4	93.1	1522.4	89.2	1393.0	81.7
3	1805.5	122.5	83.7	1683.0	93.2	1599.6	88.6	1505.4	83.4
4	1686.6	107.6	83.8	1579.0	93.6	1495.2	88.7	1405.7	83.3
5	1867.7	132.1	67.4	1735.6	92.9	1668.2	89.3	1553.4	83.2
6	1654.6	102.4	60.6	1552.2	93.8	1491.6	90.1	1433.5	86.6
7	1685.9	102.1	77.5	1583.8	94.0	1506.3	89.3	1412.5	83.8
8	1713.8	104.6	58.4	1609.2	93.9	1550.8	90.5	1429.9	83.4
9	1604.9	96.4	55.9	1508.5	94.0	1452.6	90.5	1379.6	86.0
10	1667.4	137.6	65.3	1529.8	91.7	1464.5	87.8	1341.0	80.4
11	1783.0	105.6	63.1	1677.4	94.1	1614.3	90.5	1530.6	85.8
Aver.	1732.9	114.6	69.2	1618.3	93.4	1549.2	89.4	1451.4	83.8
Vitamin B supplemented diet									
1	1886.6	119.7	70.2	1766.9	93.7	1696.7	89.9	1562.8	82.8
2	1705.9	116.1	62.3	1589.8	93.2	1527.5	89.5	1374.2	80.6
3	1805.5	117.0	63.2	1688.5	93.5	1625.3	90.0	1521.0	84.2
4	1686.6	112.0	63.2	1574.6	93.4	1511.4	89.6	1368.4	81.1
5	1867.7	122.9	66.4	1744.8	93.4	1678.4	89.9	1550.2	83.0
6	1654.6	122.4	55.7	1532.2	92.6	1476.5	89.2	1397.1	84.4
7	1685.9	106.9	64.7	1579.0	93.7	1514.3	89.8	1391.0	82.5
8	1713.8	111.9	55.8	1601.9	93.5	1546.1	90.2	1462.3	85.3
9	1604.9	104.5	51.8	1500.4	93.5	1448.6	90.3	1359.9	84.7
10	1667.4	115.0	62.4	1552.4	93.1	1490.0	89.4	1351.5	81.1
11	1783.0	115.4	48.6	1667.6	93.5	1619.0	90.8	1505.0	84.4
Aver.	1732.9	114.9	60.4	1618.0	93.4	1557.6	89.9	1440.3	83.1

that this difference is significant. Since no significant difference was observed in the fecal energy loss, the metabolizable energy was slightly less for the deficient rats than for their pair mates on the supplemented diet.

The energy loss as body heat was computed as that portion of the energy intake not recovered in urine, feces and body gain. Although in eight comparisons among the eleven the deficient rats showed a greater heat loss than did their pair mates, the average difference for the eleven pairs was equivalent to only half of 1 per cent of the gross energy of the diet. The odds are only 13.5 to 1 that this is a significant difference.

Of the 19.6 Calories difference in average gain, in favor of the rats on the supplemented diet, 8.8 Calories is accounted for by difference in the energy content of the urine, 0.3 Calorie by difference in the feces, and the remainder by difference in the heat loss.

Data showing the utilization of food energy, and the distribution of the body gains between fat and protein, are presented in table 4. The average energy intake required to produce 1 Calorie of energy gain was 18.6 Cals. for the deficient rats and 15.4 Cals. for the control rats. In other

TABLE 4

Effect of vitamin B deficiency on the utilization and distribution of energy in body gains

PAIR NO.	VITAMIN B DEFICIENT DIET						VITAMIN B SUPPLEMENTED DIET					
	Energy of body gain						Energy of body gain					
	Total	Per cent of metabolizable	As fat		As protein		Total	Per cent of metabolizable	As fat		As protein	
	Cals.		*Cals.*	*Per cent of total*	*Cals.*	*Per cent of total*	*Cals.*		*Cals.*	*Per cent of total*	*Cals.*	*Per cent of total*
1	93.9	5.6	25.0	26.6	68.9	73.4	133.9	7.9	45.4	33.9	88.5	66.1
2	129.4	8.5	58.4	45.1	71.0	54.9	153.3	10.0	75.3	49.1	78.0	50.9
3	94.2	5.9	12.4	13.2	81.0	86.8	104.3	6.4	25.7	24.6	78.6	75.4
4	89.5	6.0	31.1	34.7	58.4	65.3	143.0	9.5	68.3	47.8	74.7	52.2
5	114.8	6.9	32.9	28.7	81.9	71.3	128.2	7.6	38.9	30.3	89.3	69.7
6	58.1	3.9	−2.5	−4.3	60.6	104.3	79.4	5.4	5.2	6.5	74.2	93.5
7	93.8	6.2	25.2	26.9	68.6	73.1	123.3	8.1	63.3	51.3	60.0	48.7
8	120.9	7.8	46.3	38.3	74.6	61.7	83.8	5.4	15.1	18.0	68.7	82.0
9	73.0	5.0	7.0	9.6	66.0	90.4	88.7	6.1	27.4	30.9	61.3	69.1
10	123.5	8.4	51.7	41.9	71.8	58.1	138.5	9.3	71.7	51.8	66.8	48.2
11	83.7	5.2	7.9	9.4	75.8	90.6	114.0	7.0	31.8	27.9	82.2	72.1
Aver.	97.7	6.3	26.9	24.6	70.8	75.4	117.3	7.5	42.6	33.8	74.7	66.2

words, on the same energy intake, rats which received a diet deficient in vitamin B required 21 per cent more energy to produce one unit of energy in body gain than did the rats which received the supplemented diet.

A more specific influence of vitamin B deficiency as affecting energy metabolism is the increased carbon-nitrogen ratio (table 6) in the urine. Similar effects have been observed by Bickel ('24), and by Kon ('29).

In harmony with the higher ratio of carbon to nitrogen in the urine of the rats on the deficient diet was their consistently lower temperature. The body temperature of the rats which received the supplemented diet varied between 99.7°F. and 100.9°F.—which appears to be normal. The rats on the deficient diet, however, varied in temperature between 97.8°F. and 99.9°F., and averaged 1.2°F. below the temperature of the other group.

It is impossible to ascribe definite significance to the difference between the computed heat losses of the two groups of rats, but the low body temperature of the rats on the vitamin B deficient diet, associated with a high energy value and high carbon to nitrogen ratio in the urine, suggest that this deficiency exercises a fundamental depression of the oxidative processes of the organism.

In any case the fact remains that the vitamin B deficiency lowered the energy value of the diet—a result which supports the idea of Forbes ('32) that the maximum energy values of individual foods are not realized unless they are fed in nutritively complete rations.

NITROGEN UTILIZATION

The data of table 5 indicate a slightly greater apparent digestibility of nitrogen by the deficient rats as compared with the controls—averaging 90.21 per cent for the deficient rats and 89.43 per cent for those on the supplemented diet. This deviation, averaging 0.78 per cent in the percentage digestibility, seems very slight, but its calculated probable error is $\pm$ 0.19, and, according to Student ('08), the odds are 75 to 1

that such a difference is significant; also the digestibility of the deficient diet was apparently greater than that of the supplemented diet in nine cases among the eleven. This unexpected result may have been due to differences, 1) in digestive secretions and residues from the same, 2) in bacterial growth and residues, or 3) in completeness of absorption of digested nutriment as a result of differences in rate of passage of food residues along the alimentary tract.

TABLE 5

Effect of vitamin B deficiency on the digestibility and utilization of food nitrogen

PAIR NO.	NITROGEN INTAKE	VITAMIN B DEFICIENT DIET				VITAMIN B SUPPLEMENTED DIET			
		Nitrogen in feces	Nitrogen digested		Nitrogen intake recovered as body gain	Nitrogen in feces	Nitrogen digested		Nitrogen intake recovered as body gain
	Gm.	*Gm.*	*Gm.*	*Per cent of intake*	*per cent*	*Gm.*	*Gm.*	*Per cent of intake*	*per cent*
1	12.56	1.31	11.25	89.6	16.08	1.17	11.39	90.7	20.94
2	11.58	1.22	10.36	89.5	17.70	1.25	10.33	89.2	19.52
3	12.12	1.19	10.93	90.2	19.31	1.25	10.87	89.7	18.40
4	11.60	1.17	10.43	89.9	14.74	1.31	10.29	88.7	18.19
5	12.58	1.21	11.37	90.4	19.55	1.36	11.22	89.2	20.35
6	11.42	1.06	10.36	90.7	15.59	1.25	10.17	89.1	18.74
7	11.39	1.05	10.34	90.8	17.03	1.14	10.25	90.0	13.78
8	11.54	1.12	10.42	90.3	19.15	1.19	10.35	89.7	17.85
9	10.95	1.09	9.86	90.0	16.99	1.25	9.70	88.6	15.98
10	11.29	1.19	10.10	89.5	17.54	1.17	10.12	89.6	16.65
11	12.04	1.00	11.04	91.7	18.69	1.31	10.73	89.1	20.35
Aver.	11.73	1.15	10.59	90.2	17.49	1.24	10.49	89.4	18.25

St. Julian and Heller ('31) found no effect of vitamin B deficiency on digestibility of protein.

The percentage of the intake nitrogen recovered as body gain is dependent on the total gain of nitrogen by the animal during the experimental period, since the nitrogen intake was the same for both rats of a pair. The data in table 5, showing the percentage of nitrogen intake present in the body gain, are based on the computed gains of nitrogen reported in table 2. The portion of intake nitrogen recovered as body

gain averaged 18.25 per cent for the control rats and 17.49 per cent for the deficient rats, this difference being not statistically significant.

TABLE 6

Effect of vitamin B deficiency on the carbon-nitrogen ratio in urine

PAIR NO.	VITAMIN B DEFICIENT DIET			VITAMIN B SUPPLEMENTED DIET		
	Carbon	Nitrogen	C: N	Carbon	Nitrogen	C: N
	Gm.	*Gm.*		*Gm.*	*Gm.*	
1	7.54	8.65	0.87	6.37	8.33	0.76
2	6.00	7.92	0.76	5.72	7.77	0.74
3	7.80	8.02	0.97	5.45	8.26	0.66
4	7.28	8.22	0.89	5.95	7.55	0.79
5	5.89	8.15	0.72	5.71	8.17	0.70
6	5.24	7.74	0.68	5.04	7.59	0.66
7	6.97	7.83	0.89	5.67	8.38	0.68
8	5.19	7.61	0.68	4.78	7.71	0.62
9	4.60	7.51	0.61	4.34	7.27	0.60
10	5.65	7.76	0.73	5.40	7.85	0.69
11	5.19	8.11	0.64	3.85	7.81	0.49
Aver.	6.12	7.96	0.77	5.30	7.88	0.67

SUMMARY AND CONCLUSIONS

The effects of vitamin B deficiency on the utilization of food energy and protein were studied in growth, metabolism and body analysis experiments, by the paired feeding method, with the albino rat.

Observations were made, 1) of the dry matter, ether extract, nitrogen and energy in the bodies of the experimental animals, 2) of the nitrogen, carbon and energy of the urine, 3) of the nitrogen and energy of the feces, and 4) of the body temperatures.

The effects of vitamin B deficiency were: 1) a specific depressing effect on appetite, but no certainly significant influence on gain in weight per unit of food; 2) a decided decrease in the quantities of fat and energy gained; 3) a lower body temperature; 4) a slight increase in digestibility of protein, but no effect on the digestibility of energy-producing nutriment; 5) a diminished efficiency of utilization of metabo-

lizable energy for body gain; 6) an increased energy outgo, as urine and heat, and an increased ratio of carbon to nitrogen, in the urine; and 7) an apparent depression of the oxidative processes of the organism.

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THE FAT-SOLUBLE VITAMINS AND DENTAL CARIES IN CHILDREN [1]

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To test the effect on the progress of dental caries of fat-soluble vitamins, a group of nearly 500 seventh grade pupils was examined minutely, by clinical and x-ray exposure of every tooth. Approximately half of the number were then given for over a year extra vitamins A and D in tablet form (Squibb's Adex). The daily dose for each child was not less than 6000 U.S.P. vitamin A units and 1400 Steenbock units of D. Frequent visits by a social worker assured their consumption superimposed on the regular home diet. The other half of the children from the same social stratum served as controls. The experimental group finished with 147 children, the control with 171. The average age and sex distribution was the same in both. At the final examination, equally thorough with the first, the experimental group showed no improvement in caries incidence as judged by: 1) average increase in percentage of affected teeth; 2) average percentage increase in number of cavities; 3) percentage increase in 'average caries figure,' based on degree of involvement. Without otherwise improving the diet, the administration of concentrates of vitamin A and D to children of this age (14 at end) and within a period of 1 year seems to have no appreciable effect on the carious process in teeth already erupted or which erupt during the experimental period.

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REVIEW OF LITERATURE

Considerable difference of opinion still exists as to the value, in the diet of man and the lower animals, of an adequate supply of the fat-soluble vitamins A and D. In recent years it has become more or less generally admitted that these food factors do play an important role in the development of normal tooth and bone structures. The argument has centered more particularly upon the question as to whether or not these vitamins are of importance in the prevention or retardation of the progress of dental caries. It seems desirable, therefore, to preface the report of the present investigation with a brief résumé of the findings of the numerous other workers in this field.

In the earlier work vitamins A and D were studied together as vitamin A. Since their differentiation in 1921, the effects of each have been studied specifically and much work has been devoted to the study of their influence upon the calcification of the bones and various tooth structures.

As the factor which induces calcification, vitamin D has received the greatest amount of attention in this regard. Some work has, however, been done in relation to the effects of a deficiency of the A factor upon the dental tissues. As a result of extensive experimental work on the two vitamins, Mellanby ('30) is of the opinion that it is vitamin D which is largely responsible for the normal calcification of the dental and osseous tissues, whereas vitamin A primarily controls the development of the soft peridontal tissues. Bloch ('31) believes with Mellanby that vitamin A has no direct influence on the calcification of the teeth. On the other hand, Marshall ('27) has reported that a diet deficient in vitamin A fed to pregnant animals and puppies produced not only abnormal growth of the jaws and alveolar processes, and resulting irregularity and delayed eruption of the teeth, but also structurally defective enamel and dentine. Marshall ('28) has also demonstrated the development, after maturity, of caries in rats said to be due to vitamin A deficiency. Wolbach and Howe ('33), in a study of the effects of vitamin A

deficiency on rodents, have reported defective enamel structure and gross deformities in the incisors resulting from absent or deficient dentine formation. They consider that these effects together with the formation of denticles, pulp bone and cementicles "may reasonably be regarded in the human as vitamin A deficiency possibilities." Somewhat similar findings have been reported by Shibata ('31). Hess and Abramson ('31) do not attribute dental caries to a lack of vitamin A and Knowlton ('30) has reported failure to produce caries in young rats fed diets deficient in this vitamin.

Animal experimentation bearing upon vitamin D in relation to tooth structure and dental caries has been quite extensive. Perhaps the most noteworthy is that of Mellanby. In dogs, rats and rabbits she has demonstrated defective and poorly calcified dentine with numerous interglobular spaces which still persisted when vitamin A was added to a basal diet deficient in A and D. Furthermore, poorly calcified teeth were produced in the offspring by feeding the mother on a diet deficient in vitamin D. These findings led Mellanby to the conclusion ('29) that the anti-rachitic vitamin ranks first in importance in calcifying processes; and in view of the fact that the foods containing it are relatively few and expensive, a deficiency of this factor is more probable in the average diet than a deficiency of calcium and phosphorus great enough to interfere materially with the normal development of the dental tissues. In spite of the marked pathological changes in the dental tissues, attempts to produce caries in dogs failed. Some dentine softening, but no enamel or cement caries was found in rabbits. Only occasionally gross macroscopic lesions were found in the teeth of rats on a low vitamin D diet, but even in these, histological examination did not always disclose bacteria in the dentinal tubules.

Feeding the McCollum rachitogenic diet 3143 to rats for 7 to 42 days from the age of weaning, Becks and Ryder ('31) reported pathological changes in the dentine similar to those spoken of by Mellanby. These changes increased in extent and severity as the feeding period was prolonged. These

authors believe that the defective calcification of the dentine in rickets is primarily due to a progressive degeneration of the odontoblasts. On the same rickets-producing diet, Kronfeld and Barker ('32) found similar pathologic disturbances in the calcification of the teeth and bones and further reported that these changes may be prevented and influenced by the administration of a vitamin D preparation such as viosterol.

Corresponding disturbances in the calcifying mechanism on various rachitogenic diets have been reported by Shibata and other workers.

With the administration of vitamins A and D in the form of cod liver oil, Orban ('27) has reported a decided increase in growth of the incisors of rats. Karshan ('29) has been able to produce only slight variations in the phosphorus, calcium and total ash content in the incisor teeth of rats on rachitic diets causing marked changes in the composition of the bones.

Somewhat conflicting results have been obtained by various investigators who have attempted to produce experimental caries in animals maintained for periods upon diets deficient in vitamin D. Blackberg and Berke ('32 a) reported no enamel or dentine changes in the teeth of rats after 25 days on a strictly rachitogenic diet, but marked inflammation and degenerative changes in the pulp, occurring consistently. In more advanced stages of rickets dentine and enamel changes were observed, characterized by an increased amount of predentine and faulty calcification. In later work ('32 b) feeding 5-week old rats a modified Steenbock and Black diet no. 2965, the same investigators reported 33.3 per cent incidence of caries in the rachitic group, 100 per cent incidence in a group receiving excess viosterol and complete freedom from caries in a control group receiving the basal ration with the addition of a therapeutic amount of vitamin D.

Agnew ('33) has reported the development of caries in seventy out of seventy-one rats fed a diet low in vitamin D and phosphorus for periods of from 2 to 7 months. It is admitted that this diet was low in other minerals and vitamins

and the proteins were not of good quality. Moreover, it has been demonstrated elsewhere that there is a possibility of the initiation of the caries process by fracture through the agency of the hard corn particles which comprise a large proportion of the diet. Agnew believes that in the rat the role of vitamin D is of less importance than that of phosphorus. The results of his experiments would seem to justify this conclusion, but the work should be repeated in view of the inconsiderable number of animals in some of the experimental groups.

The work of Lilly ('32), of Knowlton ('30), and of Hoppert, Webber and Canniff ('32) would appear to indicate that a lack of vitamin D in the diet is not sufficient to produce caries, and that the administration of liberal amounts of this vitamin will not appreciably retard the decay of the teeth.

In view of the differences in metabolism, in the morphology of the teeth, and in the type of lesion produced, it is difficult to evaluate the results of this animal experimentation and to know just how far we are justified in applying these results to man. Much valuable information has been obtained from laboratory animals. We know that specific alterations or deficiencies in the diet will cause marked structural changes in the dental tissues. It has yet to be demonstrated that identical changes are brought about in the teeth of man during the development period or afterward. Furthermore, it has never been conclusively proved that the condition of hypoplasia, either microscopic or macroscopic, will cause a predisposition to dental decay. The difficulties of satisfactorily controlling such experiments on the human subject have necessarily limited the amount of direct experimentation in this field. Nevertheless, much valuable work has been accomplished.

It must be admitted that there is much evidence to support the hypothesis advanced by Mellanby and others that the control of dental caries is definitely correlated with vitamin D and calcium and phosphorus metabolism. Yet it is far from adequately established. The earlier work of Mellanby, Pattison and Proud ('24, '26, '28) suggested that the addition of

fat-soluble vitamins, principally D, retarded the development of carious cavities and brought about the arrest of active caries. This work was criticized on the score that the experimental period (7 months) was too short and that the children were all tuberculous and therefore already subject to abnormal calcium metabolism. Further work by the same investigators ('31) only served to confirm the earlier results. In the Sheffield investigation it was found that the percentage increase in degree of caries in the groups receiving supplements of olive oil, cod liver oil and radiostol, respectively, was 62.13, 10.28 and 6.57, while the corresponding figures in the Birmingham investigation were 45.85, 9.81 and 10.12. The conclusion was therefore reached that the incidence and rate of progress of caries in teeth already erupted can be lessened by the addition of fat-soluble vitamin D to the diet, inasmuch as the antirachitic vitamin is common to both the apparently effective substances.

The investigations of Weston Price ('32) point to the improved dental condition being due to general dietary improvement rather than to the addition of any specific factor. Price has reported the "control of dental caries to 90 per cent efficiency in even the worst cases" following the addition of fat-soluble activators coming from butter and cod liver oil with reinforcements of the minerals in milk and the mineral content of selected cereals. From 6 months' observation he reports ten times as many cavities in those not receiving activator concentrates.

The findings of McBeath ('33) would suggest that the dietary control of dental caries was limited in duration after the withdrawal of protective diets. He found quartz-mercury lamp radiation more effective than either viosterol or cod liver oil. It is difficult to reconcile these findings with the observation of many investigators in relation to the geographical distribution of dental caries. The disease is rampant in many tropical countries where the production of vitamin D through skin irradiation is at a maximum. Jones, Larsen and Pritchard ('30) report general disintegration of the teeth

of infants and small children in Hawaii despite a plentiful supply of vitamins, sunshine, cod liver oil, eggs, fruit juices and milk. Rickets is practically unknown in South Africa, yet 93 per cent of the children are said to have caries (Friel, Irel and Shaw, '31). From an experimental study of the teeth of children who had developed rickets in infancy, as compared with the teeth of normal children, both groups being closely observed during infancy and again at 6 or 7 years of age, Hess and Abramson ('31) have concluded that vitamin D is not the primary factor in the development of dental caries. Toverud and Toverud ('31) agree with Mellanby and others in reporting the occurrence of thin poorly calcified enamel, and interglobular spaces in the dentine following calcium, phosphorus and vitamin D deficiency during foetal life and during the period of lactation. Furthermore, in an unstated number of children examined, these investigators report high caries incidence with or without hypoplasia of the enamel where the data indicate an insufficient intake of mineral salts and vitamins (particularly D) during the developmental period both before and after birth. Toverud considers the metabolic disorder giving rise to hypoplasia (formed postnatally) is more likely to be due to deficient intake of vitamin D than to deficient mineral intake.

The conclusions reached by McKay and Rose ('31) are in conflict with those to be drawn from much of the work cited in relation to hypoplasia and dental caries. These conditions were contrasted in a group of children having the undoubted stigmata and history of past rickets, with a control non-rachitic group. It was found that gross hypoplasia of the permanent dentition was present in 22 per cent of the rachitic group and in only 2.5 per cent of the control group. This would seem to indicate a definite association between hypoplasia and vitamin D deficiency; yet the percentage of carious teeth was almost identical in both groups in both the deciduous and permanent dentitions. In the opinion of the authors, this evidence "strongly suggests, if it does not prove" that a deficiency of vitamin D in early childhood with

consequent hypoplasia, either microscopic or macroscopic, is not an important controlling factor in the incidence of dental caries. Unfortunately, the groups were small and it is impossible to rule out entirely the possible influence of subsequent antirachitic treatment upon the susceptibility of the teeth to caries. Moreover, it is impossible to estimate the proportion of cases of gross hypoplasia in each group which may have been due to metabolic disturbances arising from the various exanthemata of childhood.

Wieland ('31) has made the statement that more than 80 per cent of all infants under 1 year of age show signs of florid rickets. If this could be substantiated it would indeed be of the utmost importance to discover the true relation between rickets and hypoplasia and between hypoplasia and dental caries. Reporting on 100 children from 5 to 17 years of age suffering from rickets, varying from mild to severe, Wilson and Surie ('30) state that the degree of severity of rickets and caries appeared to run parallel. Fourteen children with mild rickets showed evidence of caries in 21 per cent of cases and of hypoplasia in 85 per cent, while all of the nine children with severe rickets showed both caries and hypoplasia.

The ratio of calcium, phosphorus and vitamin D in the diet would seem to be an important factor. It is known that the effects of Ca and P deficiency are less marked when there is much vitamin D in the diet. Similarly, less vitamin D is required to prevent rickets when there is an ample supply of calcium and phosphorus. Furthermore, an excess of either calcium or phosphorus may produce rickets.

Boyd, Drain and Nelson ('29, '31), Brodsky and Schoenthal ('33) and Bunting and his associates ('30) have all reported successful control of the caries process by dietary measures. It is important to note, however, that the results of these investigators were obtained not by the omission or inclusion of any one specific factor, but rather by the adoption of a dietary which was improved with respect to its mineral and vitamin content without the addition of mineral supplements

and sometimes without superimposing therapeutic food accessories.

Even those investigators who have stressed the value of the fat-soluble vitamins have in some instances effected improvements in the diet in relation to vitamins other than A and D and at the same time increased the mineral content. In the light of our present knowledge, it would seem reasonable to conclude that a regular diet, adequate in all essential factors is the best to follow. It is obvious, from a review of the literature, that it has not yet been definitely proved that the lack of any one factor is responsible for the high incidence of dental caries at the present day. Furthermore, the functions of digestion, absorption and intermediary metabolism, are not yet fully understood.

METHOD OF INVESTIGATION

The decisive test of the efficacy of the fat-soluble vitamins in arresting or retarding the progress of dental caries must be made on man himself. The work of Mrs. Mellanby suggests that not only teeth in the process of calcification, but those already formed may be rendered less susceptible to dental caries by the administration of an ample supply of these vitamins, mainly vitamin D.

It was therefore decided to conduct an investigation in an attempt to discover whether active caries could be arrested or significantly retarded by superimposing a generous supply of the fat-soluble vitamins upon the ordinary home diets of a group of school children. In view of the fact that the caries process is conceded to be most active around the age of puberty, it was felt that the application of these principles could be best studied with a group at the age of 13 years. Accordingly, permission was obtained to carry out the work in the seventh grade of one of the public schools of Rochester. Our original examination included the whole of this grade, numbering some 500 children of both sexes and of the average age of 13 years. This number was then reduced to some 430, in order to eliminate those much above or below the

average age and those from whom complete data were unobtainable for various reasons. The nature of the investigation was outlined and the cooperation of teachers, parents and children obtained. Of the 430 children, 220 children and their parents expressed a willingness to enter the experimental group and to cooperate for the necessary length of time. The remaining 210 constituted the control group. For the selection of the experimental group the only factor taken into consideration was the enthusiasm of the parents and children in their desire to cooperate. Subsequent investigation revealed that the two groups were as nearly as could be judged of the same economic status, with approximately equal proportions of the various nationalities. Furthermore, as will be seen from the data, the original dental condition of the two groups was practically identical. No attempt was made to interfere with, or influence in any way, the regular home diet of the children.

Each child in the experimental group received a daily ration of a fat-soluble vitamin concentrate in tablet form. The tablets were physiologically standardized, the vitamins being in a form readily assimilable. Each tablet contained not less than 1000 U.S.P. units of vitamin A and 245 Steenbock units of vitamin D. The daily ration for each child was six of the tablets which would be equivalent to three teaspoonfuls of high-grade cod liver oil, reenforced with viosterol. These were delivered at the homes periodically by an assistant (Mrs. Grace Simmons, A.B.) who was employed throughout the whole course of the investigation in order to maintain constant contact with the children and parents in their homes.

It was realized that this was the crucial factor in the investigation and our thanks are due to Mrs. Simmons for her enthusiasm and untiring efforts in this direction.

As is always the case in an investigation of this nature, it was inevitable that there should be considerable defection from the group for various reasons. Absence at one or other of the examinations or transfer from the school was accountable in part. Furthermore, if any signs of flagging interest

or irregularity in taking the concentrates were observed at any time during the course of the experiment, these children were immediately eliminated from the group. This was done in every case before the final examination and regardless of the dental condition. Although this action depleted the number of experimentals by approximately one-third, it assured us of a thoroughly reliable group. It was found that in the final assessment 147 children remained in the experimental group and 171 in the control group.

Three detailed dental inspections were made; the first in October, 1932, the second in May, 1933, and the third in December, 1933. Individual charts were used, the actual location and extent of each cavity or filling being recorded and additions made on attached charts at subsequent examinations. Cavities and fillings were differentiated by the use of different colored markings. Each child was submitted to a thorough prophylactic cleaning before examination by mouth mirror and explorer. A complete radiographic survey of every mouth was made in the first and final examinations. The discovery by this means of numerous incipient interproximal cavities at or just below the contact point, and consequently quite beyond the reach of the finest explorer proved the x-ray check-up to be not only advisable, but indispensable in investigations of this type. At the same time plaster of Paris models were made of the teeth in both upper and lower jaws for use in another series of observations.

The number of deciduous teeth remaining in the mouth at this age was very small and those still present had passed their allotted term. Although a record was kept of the condition of these teeth, it was therefore deemed advisable to disregard them entirely in our caries figure computations. All extracted teeth were regarded as having been carious except those which were known to have been lost through trauma or extracted for aesthetic or orthodontic purposes. In order to arrive at the total caries figure for each mouth and the average caries figure for the two groups, it was necessary to classify each cavity according to the extent of

the caries process. In the case of multiple caries in one tooth the summation of those numbers assigned to the individual cavities would give the caries figure for the tooth. Adding the caries figures for each tooth would give the total caries figure for the mouth. This divided by the number of erupted teeth would thus yield the average caries figure for the mouth.

It was therefore decided to adopt a modification of the method employed by Mellanby and assign numerical values as follows:

1. Initial caries including softened or discolored pits and fissures giving lodgment to a fine explorer; approximal caries subsequently verified by radiograph, but not freely accessible and small carious points in any part of the tooth.
2. Freely accessible approximal cavities and small open cavities involving less than one-fourth of the tooth.
3. More extensive caries involving more than one-fourth and less than two-thirds of the crown.
4. Caries involving from two-thirds to complete destruction of the crown.

Further data collected during the course of the investigation have been reported elsewhere.

PRESENTATION OF DATA AND DISCUSSION

It must be admitted at the outset that in order to have a satisfactory criterion of the arrest or retardation of the caries process in any given mouth or group, the rate of progress prior to the commencement of the experimental period should, theoretically, be specifically noted. However, this observation would be impracticable, or at least unreliable, for the reason that the relative increase in caries incidence is known to vary considerably as the age of the patient increases. Furthermore, it is recognized that the susceptibility to caries of the individual teeth varies. In the course of 12 months, which would be the minimum time required to make such an observation, the combination of teeth in any individual mouth may be greatly changed. It would therefore seem that the preliminary observations of this nature would be worthless.

The composition of the two groups retained for the final inspection is set out in table 1. It will be seen that the number of females in both groups was the same while there were more males in the control than in the experimental group. In view of the close agreement between the initial and final average caries figures for the male and female members of both groups, it is felt that this discrepancy cannot constitute an appreciable source of error. The average age of the controls and experimentals was very similar and extremely uniform. The children used in Mrs. Mellanby's investigations were between 5 and 14 years of age, the average being under 10 years.

TABLE 1

GROUP	NUMBER MALES	NUMBER FEMALES	TOTAL NUMBER	AVERAGE AGE AT END OF EXPERIMENT
I. Control	92	79	171	13 years 11 months
II. Experimental	68	79	147	13 years 10 months
Total both groups	160	158	318	

TABLE 2

GROUP	EXAMINED	TOTAL ERUPTED TEETH	TEETH ERUPTED DURING EXPERIMENTAL PERIOD	NUMBER DECAYED	PER CENT DECAYED
I. Control	first	4501			
	final	4681	180	106	58.89
II. Experimental	first	3763			
	final	3968	205	122	59.50

Consequently, her observations dealt largely with deciduous teeth; and the daily ration of cod liver oil was varied arbitrarily according to the age of the child.

Table 2 shows the total number of teeth erupted at the first and final inspections, respectively, in the control and experimental groups. In the fourth column will be seen the number of fresh teeth erupted during the experimental period. The next two columns indicate, respectively, the number and percentage of the newly erupted teeth which had already decayed. The percentages in the two groups closely parallel each other. It is noteworthy that such a high proportion (almost 60 per cent) of these teeth so recently erupted were already defective.

From a study of table 3 it is apparent that there is no significant difference between the experimental and control groups in the incidence of dental caries computed on the basis of the number and percentage of individual teeth affected at the first and final examinations. Comparisons made on this basis are apt to be erroneous or misleading. This is

TABLE 3

GROUP	NUMBER CASES	EXAMINED	TOTAL TEETH ERUPTED	TEETH CARIOUS OR FILLED	PERCENTAGE CARIOUS OR FILLED	INCREASE IN PERCENTAGE OF AFFECTED TEETH
I. Control	171	first	4501	2507	55.47	
		final	4681	2866	61.24	5.77
II. Experimental	147	first	3763	2084	55.38	
		final	3968	2407	60.66	5.28

TABLE 4

GROUP	ADDITIONAL CAVITIES SINCE FIRST EXAMINATION		TOTAL	NUMBER CAVITIES FIRST EXAMINATION	PER CENT INCREASE	NEW CAVITIES PER MOUTH	TOTAL
I Control	upper	690				upper 4.04	
	lower	468	1158	3197	36.22	lower 2.73	6.77
II Experimental	upper	659				upper 4.48	
	lower	447	1106	2526	43.78	lower 3.04	7.52

TABLE 5

GROUP	EXAMINED	AVERAGE CARIES FIGURE	INCREASE IN AVERAGE CARIES FIGURE	PERCENTAGE INCREASE IN AVERAGE CARIES FIGURE
I. Control	first	1.024		
	final	1.359	0.335	32.71
II. Experimental	first	0.991		
	final	1.323	0.332	33.50

well illustrated by comparing this table with tables 4 and 5. There is no doubt that the number of cavities present is a better criterion for the true dental condition in relation to the caries process, than is the number or percentage of defective teeth.

These data are set fourth in table 4, together with the percentage increase over the original number of cavities and the average number of fresh cavities per mouth. A still more

reliable method for arriving at a true caries index for any mouth or group is the method, outlined earlier, for computing the average caries figure and taking into account the extent of the caries process as well as the number of carious points. The results of these calculations have been summarized briefly in table 5. It will be seen that the original average caries figure for the group receiving the fat-soluble vitamin concentrates was quite comparable to that of the control group, the difference being approximately 3 per cent. The average increase in caries figure for the two groups was almost identical; and the percentage increase in the average caries figure showed a very small variation, well within the limits of experimental error. Only fifteen children showed no increase in the caries figure between the first and final inspections. Of these, seven were in the control group and eight in the experimental group. None of these showed absolute freedom from new cavities, the apparent arrest being due to the eruption of new teeth during the experimental period, with at the same time only a slight increase in caries incidence.

It was recognized that in experimental work of this nature the personal equation should be eliminated in so far as it is possible. It was felt that a knowledge of the groupings of the children might unconsciously influence the findings of the operators to some extent. Therefore, at the final inspection care was taken that the operators were quite unaware as to whether the child being examined was in the control or the experimental group. Furthermore, the first examination was conducted before the partition was made and this was eventually effected regardless of the dental condition.

The radiographic survey proved an invaluable aid. Our experience in this regard leads us to the conclusion that, although it adds materially to the work involved in such an investigation, the Roentgen-ray check-up is quite indispensable if reliable results are to be obtained. This fact is obvious from a study of table 6. Of the total of 2264 additional cavities which developed subsequent to the first examination, no fewer than 620 would have been overlooked even with the most

careful oral examination. All of them were located in positions at the contact point quite inaccessible to the finest explorer. The table indicates that 28.67 per cent of fresh cavities in the control group and 26.04 per cent in the experimental group could be revealed only by the x-ray. This is an increase of 37 per cent over the original number of 1644 new cavities found with mouth mirror and explorer. In many cases the radiograph disclosed no additional cavities, whereas in others the apparent increase in the caries figure was increased as much as 200 per cent.

An interesting point brought out by this investigation was the continued tendency for those children showing an originally low caries incidence to maintain a lower absolute increase

TABLE 6

GROUP	TOTAL ADDITIONAL CAVITIES SINCE FIRST EXAMINATION	TOTAL	NUMBER REVEALED ONLY BY X-RAY SURVEY	TOTAL	PER CENT REVEALED ONLY BY X-RAY
I Control	upper 690		upper 173		
	lower 468	1158	lower 159	332	28.67
II Experimental	upper 659		upper 139		
	lower 447	1106	lower 149	288	26.04
Total (2 groups)	2264		620		27.35

in the average caries figure than those with a high caries figure at the beginning of the experimental period.

In the whole series there were 68 children with a caries figure below 0.74 and averaging 0.56 and sixty-eight with a caries figure above 1.24 and averaging 1.52. The respective increases in the average caries figure for the two groups was 0.28 and 0.44. Whether this is due to the influence of heredity, family diet or other factors, it is impossible to say. It is of interest to note that Hanke observed the same phenomenon among children on identical diets.

The caries figure of hypoplastic teeth showed no greater tendency to increase than that of teeth with no evidence of gross hypoplasia.

SUMMARY AND CONCLUSIONS

An experiment was conducted to test the effect of fat-soluble vitamin concentrates upon the progress of dental caries

The concentrates were given in tablet form, the daily ration for each child containing not less than 6000 U.S.P. units of vitamin A and 1470 Steenbock units of vitamin D in the form of viosterol.

The daily ration was superimposed upon the ordinary diet of the child, no attempt being made to interfere with the food intake in any way.

The experimental group comprised 147 children of both sexes and of the average age of approximately 13 years at the beginning of the experimental period.

A comparable group of 171 controls receiving no vitamin concentrates was kept under observation.

The first and final inspections were made, respectively, in October, 1932, and December, 1933.

It was felt that children of this age would be ideal for such a study, in view of the fact that the caries process is most active around the age of puberty.

No beneficial effect could be observed in respect to the incidence of dental caries when the two groups were compared, as judged by 1) the average increase in percentage of affected teeth, 2) the average percentage increase in the number of cavities, or in the number of cavities per mouth, 3) the percentage increase in the average caries figure.

Furthermore, the percentage of teeth which erupted and became carious subsequent to the first examination was just as high in the group receiving concentrates as in the control group.

The caries process was equally as active between the second and third inspections as between the first and second.

One therefore feels justified in drawing the conclusion that without otherwise altering the diet, the administration of vitamins A and D to children of this age and within the period of this investigation, has no appreciable effect upon the rate of progress of the caries process in teeth already erupted or which erupt during the experimental period.

The necessity for radiographic surveys in all studies of this nature is evidenced by the data presented.

Of some interest is the demonstrated tendency for children showing an originally low caries incidence to maintain a lower absolute increase in the average caries figure than those with a high caries figure at the beginning of the experimental period.

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THE USE OF THE METHOD OF PARTIAL REGRESSION IN THE ANALYSIS OF COMPARATIVE FEEDING TRIAL DATA.[1] PART II

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INTRODUCTORY

In a previous paper (Crampton and Hopkins, '34) it was shown that the method of partial regression offers attractive possibilities as an aid to the interpretation of comparative feeding trial data. As a means of correcting live weight gains for effects of varying initial weights and feed consumption of pigs, it appears to be more satisfactory than the methods in common use. It was pointed out that the analysis might be made on the final weights of the animals equally well as on gains in weight, correcting them, as was done in the case of gains, by means of the partial regression formula for differences in initial weight and feed eaten, for, after all, the calculation of gains is but an attempt to correct for variations in initial weight. If all pigs of a trial were of the same weight at the start and consumed equal quantities of feed, a comparison of final weights would be the simplest analysis possible. It would seem, therefore, that if the effects of differences in the initial weights of the pigs and of differences in their feed consumption could be efficiently corrected for by partial regression, the final weights of the pigs would often be the logical data on which to base statistical analysis of such trials.

[1] Contribution from the Faculty of Agriculture of McGill University, Macdonald College, P. Q., Canada. Journal series no. 37.

The final weights of the four series of pigs referred to in the previous paper will, therefore, be treated in this way, in order to illustrate its possibilities, after which the application of the method to comparative trials involving several differently treated lots will be illustrated by the analysis of a further series of results, obtained in an actual experiment of this type.

ANALYSIS OF FINAL WEIGHTS

Experimental data. The experimental results used in this study were derived from the same source as those employed in the previous paper, and, excepting that in place of gains (y_1), final weights (y_2) were used, the analysis was performed by the same method.

The analysis of the variance and covariance of initial weight, feed consumption, and final weight is given in table 1.

It will be seen from table 1 that final weight is closely correlated with both initial weight and feed consumption. This is also true of gains, excepting in the D series, in which gains do not show a significant correlation with initial weight.

Regression analysis. The partial regression coefficients (computed as explained in part I of this series), of final weight on initial weight and feed consumption for the four series are given in table 2.

The standard errors of the various coefficients are also indicated, together with the ratio of the coefficients to their standard errors and the probability of obtaining such values by chance sampling from a population in which the attributes in question are uncorrelated.

It will be noted from table 2 that all b_2 coefficients are highly significant, as are the b_1 for series A, B, and C.

The regression of final weight on initial weight (b_1) increases with increasing age of pig from 0.791 to 1.116. This means that in series A, a pig 10 pounds heavier than another at the outset will, on the average, be only 7.9 pounds heavier at the end of a 30-day feeding period, feed consumption being equal. In series C, on the other hand, differences in weight

at the outset are magnified 1.12 times at the end of the period, in the absence of differences in feed consumption.

TABLE 1

Standard deviation of and correlation between initial weight, feed eaten, and final weight of swine of different age groups

VARIABLES	MEAN	VARIANCE OR CO-VARIANCE	S.D.	C.V.	r	r NECESSARY FOR P = 0.01
Series A (N = 167)						
Initial weight	28.6	42.59	6.53	22.83	....	....
Feed eaten	106.5	408.66	20.22	18.99	....	....
Final weight	66.8	156.66	12.52	32.22	....	....
Initial weight × final weight ($x_1 y_3$)	....	66.08	....	....	0.81	0.25
Initial weight × feed ($x_1 x_2$)	....	80.39	....	....	0.61	0.25
Feed eaten × final weight ($x_2 y_3$)	....	228.30	....	....	0.90	0.25
Series B (N = 69)						
Initial weight	64.1	155.51	12.47	19.45	....	....
Feed eaten	391.7	2411.22	49.10	12.54	....	....
Final weight	158.7	493.48	22.21	13.99	....	....
Initial weight × final weight ($x_1 y_3$)	....	226.75	....	....	0.82	0.32
Initial weight × feed ($x_1 x_2$)	....	402.30	....	....	0.66	0.32
Feed eaten × final weight ($x_2 y_3$)	....	900.38	....	....	0.83	0.32
Series C (N = 89)						
Initial weight	155.5	471.40	21.71	13.96	....	....
Feed eaten	240.8	802.25	28.32	11.76	....	....
Final weight	206.7	758.41	27.54	13.32	....	....
Initial weight × final weight ($x_1 y_3$)	....	571.58	....	....	0.96	0.28
Initial weight × feed ($x_1 x_2$)	....	344.23	....	....	0.56	0.28
Feed eaten × final weight ($x_2 y_3$)	....	490.00	....	....	0.63	0.28
Series D (N = 48)						
Initial weight	67.6	129.30	11.37	16.81	....	....
Feed eaten	514.8	4564.75	67.56	13.12	....	....
Final weight	193.1	449.92	21.21	10.98	....	....
Initial weight × final weight ($x_1 y_3$)	....	165.85	....	....	0.69	0.37
Initial weight × feed ($x_1 x_2$)	....	575.41	....	....	0.75	0.37
Feed eaten × final weight ($x_2 y_3$)	....	1200.18	....	....	0.84	0.37

The increase in final weight per unit of feed eaten (b_2) decreases markedly with increasing age of pig, from 0.40 pound weight for each additional pound of feed consumed in series A to 0.13 pound in series C.

Reduction of variance. As explained in section 3 of part I (Crampton and Hopkins, '34), the regression coefficients may be used to correct the observed final weights for variations

TABLE 2

Partial regression coefficients of final weight on initial weight (b_1) and feed eaten (b_2)

SERIES	N	INITIAL WEIGHT				FEED EATEN			
		b_1	Standard error ($s\sqrt{C_{11}}$)	$t = \frac{b}{s\sqrt{C_{11}}}$	P	b_2	Standard error ($s\sqrt{C_{22}}$)	$t = \frac{b}{s\sqrt{C_{22}}}$	P
A	167	0.791	0.0531	14.89	< 0.01	0.403	0.0177	22.77	< 0.01
B	69	0.866	0.1258	6.88	< 0.01	0.229	0.0319	7.17	< 0.01
C	89	1.116	0.2272	4.10	< 0.01	0.132	0.0340	3.88	< 0.01
D	48	0.257	0.2219	1.15	0.25	0.231	0.0374	6.17	< 0.01

TABLE 3

Variance of final weights

SERIES	OBSERVED FINAL WEIGHTS				FINAL WEIGHTS CORRECTED BY REGRESSION FOR INITIAL WEIGHT AND FEED INTAKE				RELATIVE PRECISION
	D/F	Sum of squares	Variance	Standard error	D/F	Sum of squares	Variance	Standard error	
A	166	26004.9	156.66	12.52	164	2053.6	12.52	3.54	12.5
B	68	33556.5	493.48	22.21	66	6185.8	93.72	9.68	5.3
C	88	66740.4	758.41	27.54	86	4908.6	57.08	7.56	13.2
D	47	21146.2	449.92	21.21	45	6138.2	136.40	11.68	3.3

in initial weight and feed intake. In table 3 is given a summary of the variance of, a) observed final weights and b) final weights corrected by means of the partial regression coefficients for variations in the above factors. As might be expected, this correction results in a marked increase in precision.

Table 4, showing the variance of gains of the same animals, is included for comparison. It will be noted that the final

results are the same, whether gains or final weights form the basis of the analysis, the variance and standard deviations after correcting for effects of differences in initial weight and of feed intake being identical. If the actual weights are used, the labor of calculating the gains is avoided.

TABLE 4

Variance of gains

SERIES	OBSERVED GAINS				GAINS CORRECTED BY REGRESSION FOR INITIAL WEIGHT AND FEED INTAKE				RELATIVE PRECISION
	D/F	Sum of squares	Variance	Standard error	D/F	Sum of squares	Variance	Standard error	
A	166	11134.6	67.08	8.19	164	2054.2	12.52	3.54	5.3
B	68	15468.2	227.47	15.08	66	6190.5	93.79	9.68	2.4
C	88	7625.2	86.65	9.31	86	4908.4	57.07	7.56	1.5
D	47	11634.1	247.53	15.73	45	6139.8	136.44	11.68	1.8

ANALYSIS OF RESULTS OF COMPARATIVE TRIALS

By means of the analysis of covariance (Fisher, '30, sec. 49, 1) it is possible to determine the regression of final weight on initial weight and feed consumption from the observed variance and covariance within the lots of a comparative trial. The regression coefficients may be employed as before to reduce the variance within lots, and also to make due allowance for the effects of differences in average initial weight and feed consumption between lots, thus enabling more accurate and unbiased conclusions to be drawn. To illustrate this application of the method, the results of a test recently completed at Macdonald College have been analyzed in this way and will be used as an example.

This trial involved five comparative lots of hogs fed over a period of 105 days. The pigs were allotted and started on test at 60 days of age. They were individually penned and hand full-fed throughout the trial. The comparative rations differed only in protein level, but the details of ration composition need not be given here, as it is the method of analysis rather than results of this particular trial which we wish to demonstrate.

Table 5 gives the initial and final weights of the pigs, the gain in live weight in 105 days of feeding, and the quantities of feed eaten during the test. The mean gain and final weight, actual and corrected, of each lot are also inserted.

The variance and covariance of the quantities listed in table 5 have in table 6a been analyzed into portions attributable to treatment differences, to differences between replicates, and to residual or error deviations. The required partial regression coefficients may now be estimated from the residual sums of squares and products, these representing the total covariance of initial weight, gain, etc., within lots after the elimination of treatment and replicate differences.

The partial regression coefficients of gain on initial weight (b_1) and on feed intake (b_2) thus calculated are:

$$b_1 = -0.01304; \quad b_2 = 0.24118$$

The corresponding coefficients using final weight instead of gain are: $b_1 = 0.98696$; $b_2 = 0.24118$

The analysis of variance of gains corrected for differences in initial weight and feed consumption by the regression formula is given in table 6b. The corrected sums of squares are obtained from the uncorrected values of table 6a by means of the relationship:

$$S(y - b_1 x_1 - b_2 x_2)^2 = S(y^2) - 2\,b_1 S(x_1 y) - 2\,b_2 S(x_2 y) + b_1 b_2 S(x_1 x_2) + b_1^2 S(x_1^2) + b_2^2 S(x_2^2)$$

(x_1, x_2 and y being understood to be measured from their respective means), which is applied to each line of table 6a in turn. In the case of the residuals from which b_1 and b_2 were estimated, the right member reduces to

$$S(y^2) - b_1 S(x_1 y - b_2 S(x_2 y)$$

Lot means are corrected for differences in average initial weight and feed consumption as follows:

Corrected mean $= (\overline{Y}_1 - \overline{Y}) - b_1 (\overline{x}_{11} - \overline{x}_1) - b_2 (\overline{x}_{21} - \overline{x}_2) + \overline{Y}$ when $\overline{Y}$ is the general mean (gain or initial weight),

$\overline{Y}_1, \overline{Y}_2 \text{ ——— } \overline{Y}_n$ the means of lots 1, 2, etc.,

$\overline{x}_{11}, \overline{x}_{12} \text{ ——— } \overline{x}_{1n}$ the mean initial weight (x) of lots 1, 2, etc.,

$\overline{x}_{21}, \overline{x}_{22} \text{ ——— } \overline{x}_{2n}$ the mean feed eaten (x) of lots 1, 2, etc., and

b_1 and b_2 the regression coefficients of gain or final weight on initial weight and feed eaten, respectively.

TABLE 5

Weights, gains, and feed consumption of pigs in comparative feeding trial

REPLICATE	LOT I				LOT II				LOT III				LOT IV				LOT V			
	Initial weight x_1	Feed eaten x_2	Gain y_1	Final weight y_2	Initial weight x_1	Feed eaten x_2	Gain y_1	Final weight y_2	Initial weight x_1	Feed eaten x_2	Gain y_1	Final weight y_2	Initial weight x_1	Feed eaten x_2	Gain y_1	Final weight y_2	Initial weight x_1	Feed eaten x_2	Gain y_1	Final weight y_2
1	30	674	165	195	26	699	168	194	39	708	164	203	41	716	185	226	41	831	201	242
2	21	628	156	177	24	626	180	204	34	614	156	190	35	769	195	230	36	754	189	225
3	21	661	159	180	20	668	180	200	32	733	189	221	32	733	186	218	32	722	173	205
4	33	694	167	200	35	668	166	201	35	663	138	173	34	742	201	235	35	728	193	228
5	27	713	170	197	25	707	170	195	32	607	153	185	32	624	165	197	32	646	164	196
6	24	585	146	170	26	651	161	187	35	745	190	225	35	710	175	210	36	678	160	196
7	20	575	130	150	20	672	171	191	30	637	160	190	30	742	187	217	30	763	200	230
8	29	638	151	180	31	660	169	200	29	662	172	201	28	648	177	205	28	625	142	170
9	28	632	164	192	29	769	179	208	32	609	142	174	34	628	166	200	32	710	184	216
10	26	637	158	184	27	666	191	218	25	596	155	180	26	601	165	191	26	651	149	175
Means	25.9	643.7	156.6	182.5	26.3	678.6	173.5	199.8	32.3	657.4	161.9	194.2	32.7	691.3	180.2	212.9	32.8	710.8	175.5	208.3
Corrected mean[1]			164.6	194.4			173.1	202.9			166.5	196.5			176.6	206.7			167.2	197.2

[1] See table 7.

Tables 6a, 6b, and 6c give the analysis of variance of the gains and of the final weights, respectively, both before and after correction for the effects of varying initial weight and feed intake.

It will be noted that in the analysis of both tables, the variance between replicates, corresponding to 9 degrees of freedom, has been separated from the error sum of squares. In the present instance, this variance is actually less than the expectation from the residual variance within lots, with the result that the estimated standard error from the 36 residual

TABLE 6a

Variance and covariance of gains and final weights

VARIANCE DUE TO	DEGREES FREEDOM	VARIANCE				COVARIANCE				
		Initial weights (x_1)	Feed eaten (x_2)	Gain (y_1)	Final weights (y_2)	$x_1 x_2$	$x_1 y$	$x_2 y$	$x_1 y_2$	$x_2 y_2$
Total	49	27.18	3149.95	288.05	380.29	149.76	32.53	766.15	59.71	915.90
Treatment	4	127.30	7101.23	976.63	1435.43	546.95	165.75	2352,18	293.05	2899.12
Replicate	9	50.49	3905.59	159.73	276.36	278.22	33.07	696.68	83.56	974.90
Error	36	10.23	2522.01	243.62	189.03	73.51	17.59	607.29	27.83	680.80

TABLE 6b

VARIANCE DUE TO	DEGREES FREEDOM	SUMS OF SQUARES OF GAINS		CORRECTED VARIANCE	$1/2 \log_e$	STANDARD ERROR
		Uncorrected gains	Corrected by regression			
Total	49	14114.4	4979.3	101.62		
Treatment	4	3906.5	1024.0	256.00	2.7725	
Replicate	9	1437.6	449.8	49.98		
Error	36(34[1])	8770.3	3505.8	103.12	2.3179	10.15

TABLE 6c

VARIANCE DUE TO	DEGREES FREEDOM	SUMS OF SQUARES OF FINAL WEIGHTS		CORRECTED VARIANCE	$1/2 \log_e$	STANDARD ERROR
		Uncorrected final weights	Corrected by regression			
Total	49	18634.4	4980.5	101.64		
Treatment	4	5741.7	1024.1	256.00	2.7725	
Replicate	9	2487.2	450.0	50.00		
Error	36(34[1])	10405.5	3506.2	103.12	2.3179	10.15

[1] Two additional degrees of freedom lost through calculation of two constants b_1 and b_2 from this data. Hence for error in corrected sums of squares the degrees of freedom is 34 instead of 36.

degrees of freedom in table 6a is 15.6 pounds, as compared with the value of 15.1 obtained from the total 45 degrees of freedom within lots. When significant differences between replicates do exist, however, a corresponding diminution of error is effected by the elimination of variation arising from this source. The reduction of the error variance in this way is quite legitimate, and is indeed one of the advantages to be derived from the structure of the experiment. Average differences between replicates affect all the members of a given replicate equally, and hence do not enter into treatment comparisons.

Comparison of the error variance of the uncorrected and corrected gains (or final weights) indicates the increase in

TABLE 7

Corrected mean gains and final weights from table 5

LOT	MEAN GAIN	MEAN FINAL WEIGHT
I	164.6	194.4
II	173.1	202.9
III	166.5	196.5
IV	176.6	206.7
V	167.2	197.2

relative precision effected by the correction. In the case of the gains the ratio is 10 to 23, and in the case of final weights 10 to 28. In other words, equally reliable results could be obtained from ten pigs whose gains were corrected by this method for differences in feed intake and varying initial weights as from twenty-three pigs using uncorrected gains.

Attention has already been directed to the fact that the same estimate of error is arrived at whether gains or final weights are subjected to analysis. Table 7 illustrates a further point, namely, the identity of the differences between lots obtained in each case. This identity is a necessary consequence of the gain being a particular linear function (the difference) of the initial and final weights. The calculation of gains is thus seen to contribute no increase in accuracy to the analysis.

The necessary difference. a) Test of significance.[1] Using the standard error of 10.15, the difference between two lots, of ten pigs each, necessary to indicate that treatment had a significant effect will be:

$$\frac{s\sqrt{2}}{\sigma n} \times t_{P=.05} = \frac{10.15 \times 1.414}{3.16} \times 2.262 = 10.3 \text{ lb.}$$

b) Estimation of effects of treatment. Between means showing this (10.3 lb.) or larger differences the necessary difference to cover experimental error, with odds of 19 to 1, will be—

$$\frac{10.15 \times 1.141}{3.16} \times 1.833 = 8.3 \text{ lb.}$$

since for odds of 19 to 1, t for $P = 0.1$ may now be used instead of t for $P = 0.05$.

Examination of the corrected means indicates a significant difference between lots IV and I in favor of lot IV. Deducting from the difference of 12.0 between these two means the necessary difference of 8.3 leaves a net difference in gains creditable to treatment of 3.7 pounds. We should be justified, therefore, in claiming just over 2 per cent faster gains due to treatment in lot IV as compared to lot I.

SUMMARY

A previous statistical study of the relationship between the initial weight and feed consumption of swine of four different age groups and their performance in experimental feeding trials has been extended, and further examples of the application of these relationships to the reduction of the experimental error in such trials have been given.

In all age groups a highly significant relationship between final weight and feed eaten is demonstrable, the increase in final weight per unit of additional feed consumed decreasing

[1] It has recently been shown by Cochran, (Proc. Camb. Phil. Soc., XXX, 177–191) that the ratio of the corrected 'treatment' to 'error' sum of squares will not follow exactly the Z distribution. If, therefore, it is also desired to apply the Z test of significance to the observations as a whole, a 'treatment' sum of squares must be computed by a slightly different method.

with advancing age of pig. The final weight attained is also influenced by the initial weight of the pig, except possibly in the case of young pigs fed through to market weight.

It is shown that identical results are obtained whether the gains or final weights attained are corrected for variations in feed consumption by means of the appropriate regression formula. The usual calculation of gains thus leads to no increase in accuracy, and unless of particular interest may be omitted.

The application of the method of partial regression to the analysis of comparative feeding trial data has been illustrated, using the results of an experiment recently completed at Macdonald College in which five lots of ten pigs each were fed for a period of 105 days on rations differing in protein level.

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RESULTS OF THE INGESTION OF COD LIVER OIL AND YEAST ON CALCIUM AND PHOSPHORUS METABOLISM OF WOMEN

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It is generally taken for granted that the normal adult, living in the usual urban environment with little exposure to sunshine, needs a dietary enriched with vitamin D for the regulation of calcium and phosphorus metabolism, although the proof of this supposition has not been demonstrated through the administration of cod liver oil (Hart, Tourtellotte and Heyl, '28), irradiated ergosterol (Bauer, Marble and Claflin, '32), or cod liver oil and direct irradiation of the subjects with the mercury vapor lamp (Dye, '30). Moreover, yeast with its potent source of the vitamin B complex is alleged to benefit mineral metabolism in the adult (Bogart and Trail, '22).

In view of the apparent confusion concerning the effectiveness of vitamins upon the calcium and phosphorus retention in the adult, additional information has been obtained upon the influence of cod liver oil alone and of cod liver oil and yeast on the calcium and phosphorus metabolism of three healthy young women. These women were under observation for 38 days, during which they consumed their customary diets, unrestricted in quality and quantity, and carried on their usual daily activities, thus reproducing the conditions existing under the ordinary adult business life. The standardized metabolic technic used in this laboratory was followed for weighing and sampling the diets for analyses and for quantitatively collect-

ing, preparing, and analyzing the excreta (Macy, Hunscher, Nims and McCosh, '30; Donelson, Nims Hunscher, Shukers and Macy, '31).

The initial control period consisted of a 15-day pre-experimental period divided into five 3-day metabolic balances when no changes were made in the dietary. Following immediately thereafter, 15 gm. of cod liver oil were given daily, and, after an adjustment period of 11 days, four successive 3-day experimental metabolic balances were made, during the first two of which the influence of cod liver oil alone on calcium and phosphorus metabolism was observed, and during the last two periods the effect of 10 gm. of yeast daily in addition to the cod liver oil[1] was observed.

Although subjects X, Y, and Z were taking a liberal daily diet, containing an average of 0.819, 1.094, and 1.055 gm. of calcium, respectively, 70 per cent of which was secured in the form of milk and cheese, they gave individual responses to the same diet, and during the first five metabolic balances in the pre-experimental period, X and Y were practically in equilibrium, while Z was losing on an average of 0.194 gm. of calcium per day (table 1). There were noticeable variations among the subjects in the retentions of calcium which were not always commensurate with changes in the level of intake. The administration of cod liver oil did not stimulate any definite change in metabolism, for the retentions of calcium were as variable and in approximately the same range of magnitude as they were before the supplement was made to the diet, except in the case of subject X, who showed some improvement. These negative responses of the calcium balance in adults to the administation of cod liver oil corroborate the findings of other investigators (Hart, Tourtellotte and Heyl, '28; Bauer, Marble and Claflin, '32; Dye, '30).

The fat content of the diet and the calcium balances manifest no relationship, for, in some instances, particularly in the

[1] We wish to express our thanks and appreciation to A. D. Emmett, Ph.D., and E. A. Sharp, M. D., of Parke, Davis & Co., Detroit, for the cod liver oil, and to Maurice H. Givens, Ph.D., of the Northwestern Yeast Company, Chicago, for the yeast used in this study.

TABLE 1

Daily calcium balance

PERIODS	SUBJECT X					SUBJECT Y					SUBJECT Z				
	Intake	Outgo			Balance	Intake	Outgo			Balance	Intake	Outgo			Balance
		Total	Urine	Feces			Total	Urine	Feces			Total	Urine	Feces	
Pre-experimental															
	gm.	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*gm.*
I	0.721	1.100	10.5	89.5	—0.379	0.780	0.766	17.6	82.4	+0.014	0.832	0.758	19.1	80.9	+0.074
II	1.078	0.874	16.7	83.3	+0.204	1.039	1.111	18.0	82.0	—0.072	1.084	1.418	17.0	83.0	—0.334
III	0.777	0.682	17.7	82.3	+0.095	1.494	1.514	13.8	86.2	—0.020	1.507	1.692	15.4	84.6	—0.185
IV	0.782	1.012	10.8	89.2	— 0.230	1.063	1.097	14.4	85.6	—0.034	1.045	1.461	13.9	86.1	—0.416
V	0.739	0.751	13.2	86.8	—0.012						0.805	0.915	18.7	81.3	—0.110
Average	0.819	0.884	13.8	86.2	—0.064	1.094	1.122	16.0	84.0	—0.028	1.055	1.249	16.8	83.2	—0.194
Experimental—cod liver oil															
I	0.682	0.781	22.2	77.8	—0.099	1.229	1.007	22.5	77.5	+0.222	0.993	1.349	13.6	86.4	—0.356
II	1.129	1.260	12.8	87.2	—0.131	0.896	1.108	19.2	80.8	—0.212	1.877	1.749	18.0	82.0	+0.128
Average	0.905	1.020	17.5	82.5	—0.115	1.062	1.058	20.8	79.2	+0.010	1.435	1.549	15.8	84.2	—0.114
Experimental—cod liver oil and yeast															
I	0.970	1.167	15.2	84.8	—0.197	1.475	1.732	15.0	85.0	—0.257	1.638	1.274	28.2	71.8	+ 0.364
II	0.784	0.894	18.0	82.0	—0.110						1.170	2.002	16.6	83.4	—0.832
Average	0.877	1.030	16.6	83.4	—0.153						1.404	1.638	22.4	77.6	—0.234

results of X and Y, an increase of fat in the diet was accompanied by an increase in the per cent of total outgo of calcium in the feces, whereas this was not always the rule. These observations are in accord with those of Mallon, Jordon and Johnson ('30), who concluded that "fat per se cannot be said to exercise a definite influence upon the calcium retention" in women.

The diets contained an average of 1.394, 1.543 and 1.631 gm. of phosphorus per day for X, Y, and Z, respectively (table 2). During the control period the resultant mean phosphorus retentions were + 0.299, and + 0.208, and + 0.225 gm., but after cod liver oil therapy the average retentions changed to + 0.034, + 0.420, and + 0.224 gm., and during the silmultaneous administration of yeast and cod liver oil they were —0.260, + 0.270, and —0.260 gm. for subjects X, Y, and Z, respectively. Thus it is seen that after cod liver oil alone was given there was a decrease in the phosphorus retention for the subject X, an increased retention for subject Y, and no change for subject Z. On the other hand, when cod liver oil and yeast were given together, there was a lowered phosphorus retention in all three subjects. These negative findings, resulting from the addition of cod liver oil, corroborate those of Bauer, Marble, and Claflin ('32), but are in contrast to those observed by Dye ('30). When cod liver oil was included in the diet, the calcium and phosphorus increased in the urine and decreased in the feces of subjects X and Y.

From the results obtained in twenty-seven 3-day calcium and phosphorus balances on three healthy women each of whom was under observation for 38 continuous experimental days when consuming her usual unrestricted diet and participating in her customary activities, no consistent physiologic changes were noted in the retention of calcium and phosphorus due to either cod liver oil or yeast.

TABLE 2

Daily phosphorus balance

PERIODS	SUBJECT X					SUBJECT Y					SUBJECT Z				
	Intake	Outgo			Balance	Intake	Outgo			Balance	Intake	Outgo			Balance
		Total	Urine	Feces			Total	Urine	Feces			Total	Urine	Feces	
Pre-experimental															
	gm.	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*gm.*	*gm.*	*gm.*	*per cent*	*per cent*	*gm.*
I	1.091	1.157	37.4	62.6	—0.066	1.175	1.047	55.5	44.5	+0.128	1.203	0.952	47.5	52.5	+0.251
II	1.484	0.890	42.5	57.5	+0.594	1.577	1.421	50.4	49.6	+0.156	1.581	1.412	38.0	62.0	+0.169
III	1.382	1.057	48.4	51.6	+0.325	1.838	1.501	41.3	58.7	+0.337	1.936	1.668	42.1	57.9	+0.268
IV	1.382	1.197	36.7	63.3	+0.185	1.582	1.371	51.1	48.9	+0.211	1.675	1.573	53.1	46.9	+0.102
V	1.629	1.174	36.8	63.2	+0.455						1.760	1.423	42.4	57.6	+0.337
Average	1.394	1.095	40.4	59.6	+0.299	1.543	1.335	49.6	50.4	+0.208	1.631	1.406	44.6	55.4	+0.225
Experimental—cod liver oil															
I	1.213	1.146	59.5	40.5	+0.067	1.650	1.039	53.7	46.3	+0.611	1.575	1.701	43.6	56.4	—0.126
II	1.675	1.671	42.6	57.4	+0.004	1.280	1.050	50.7	49.3	+0.230	2.224	1.651	42.7	57.3	+0.573
Average	1.444	1.408	51.0	49.0	+0.036	1.465	1.044	52.2	47.8	+0.420	1.900	1.676	43.2	56.8	+0.224
Experimental—cod liver oil and yeast															
I	1.638	1.700	51.1	48.9	—0.062	2.004	1.734	39.9	60.1	+0.270	2.080	1.803	59.7	40.3	+0.277
II	1.084	1.541	61.7	38.3	—0.457						1.507	2.305	45.2	54.8	—0.798
Average	1.361	1.621	56.4	43.6	—0.260						1.794	2.054	52.4	47.6	—0.260

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THE DETERMINATION OF VITAMIN A VALUES BY A METHOD OF SINGLE FEEDINGS

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TWO FIGURES

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Establishment of the position of carotene as precursor of vitamin A does not imply that there is a sole precursor nor that problems of the formation, bodily storage and utilization of vitamin A can be dealt with in terms of only two substances, one the precursor and the other the vitamin itself. In fact, three carotenes (not necessarily of equal potency as vitamin A precursors) are already recognized, and one should also take account of the possibility that the carotenes are not the only substances from which vitamin A can be formed.

Hence, while much is being learned from experiments in which carotene and vitamin A are estimated separately by colorimetric methods and the spectrophotometric examination of the intensity of absorption bands (e.g., Moore, '30; Coward, Dyer, Morton and Gaddum, '31), there is still need for the measurement of vitamin A values which may be due in part to the vitamin itself, in part to one or more of the carotenes, and possibly also in part to other precursors. Such measurements must remain fundamental to the studies of the problems of specificity of vitamin A reactions and of the functioning of the precursors of vitamin A; and upon such studies the further development of our knowledge of this vitamin and its nutritional significance will doubtless largely depend.

Methods now current involve the feeding of the material under investigation continuously for 3 or more weeks. This not only calls for a large expenditure of the time and attention of the investigator but also implies the assumption, which must often be doubtful and sometimes improbable, that the material under investigation will remain unchanged throughout the weeks of such a feeding period.

Such studies, for example, as of autopsy material, of the tissues of individual experimental animals, of plants or their products at definite stages of growth or ripening, or of the freshly prepared products of laboratory investigation (whether synthetic or by fractionation of natural materials) would not only be greatly facilitated but would gain in scientific value if the material to be tested could be fed promptly when ready without the necessity of an assumption that the sample in hand is stable or that the next sample will exactly duplicate it and will be ready exactly when wanted. Samples only occasionally available may have much scientific value for the study of certain specific problems.

Cammack ('25) suggested that the continuous feeding process might be replaced by using a single feeding of vitamin A containing food to experimental rats and then measuring in days the increased survival of such animals over the negative controls. Nelson, Walker and Jones ('31, '32) have reported the use of single feedings of cod liver oil to rats and the consequent gain in survival as a means of studying the ability of the animal to store vitamin A.

If a method of single feedings were shown to be practical, it would be useful in the study of foods or other materials which are available in the fresh state for a very limited time, or which are obtainable only in small quantities.

The present work is an attempt to develop such a method. As a basis of this study, carotene, which been proposed as an international unit of reference, was used. Other materials were tested in parallel with carotene and the data obtained were examined in terms of the results of carotene feeding.

EXPERIMENTAL RESULTS AND DISCUSSION

Albino rats of known nutritional history weighing 35 to 55 gm. at 21 to 28 days of age were used. They were depleted of their surplus body stores of vitamin A while maintained on vitamin A-free basal diet (Sherman and Munsell, '25); and the average weight of 162 rats at the end of the depletion period was 101 gm. The depletion time averaged 32.3 days, with 28 and 36 days as the extremes. The general experimental technic was that of Sherman and Burtis ('28) and Sherman and Batchelder ('31). The basal diet was: purified casein, 18 per cent; dried yeast, 10 per cent; Osborne and Mendel salt mixture, 4 per cent; sodium chloride, 1 per cent; cornstarch, 67 per cent. Vitamin D was incorporated in the diet in the form of viosterol in such an amount as to give antirachitic activity equivalent to the presence in the basal diet of 3 per cent of a cod liver oil having 100 vitamin D units per gram.

Carotene (m.p. 172° uncor.) was obtained from the British Drug Houses, Limited. It was administered orally in peanut oil solution (the peanut oil was shown by feeding tests to be inactive) using a glass syringe graduated in one hundredths of a cubic centimeter. The carotene solution was stored in a dark bottle at $0° \pm 1°$C., and colorimetric readings, using potassium dichromate solution as a reference, were made frequently to check any change in color, which would indicate a change in potency of the carotene.

At the end of the depletion period, the experimental rats were divided into groups of comparable weight, sex, and litter; one group served as negative controls, and others received a single feeding of carotene or other material. Weekly weighings of all rats were made during the experimental period which lasted from the time when the single feeding was given until death.

Carotene was given in single feedings of 28 γ, 56 γ, 112 γ (γ = 0.001 mg.) and the growth responses and survival period are shown in figure 1. Broken lines indicate the death of one or more animals and the end point of each curve represents the average weight and survival in days for that group

of rats. It is observed that a large single feeding of carotene causes increased growth and survival over that obtained by the negative controls, and increasing the quantity of the single feeding gives further increases in growth and survival. The survival periods were: negative controls 19.2 ± 0.6 days; after feeding .28 γ carotene 25.3 ± 0.6 days; after 56 γ carotene 31.7 ± 0.7 days; and after 112 γ carotene 38.1 ± 1.4 days. The increase in height of the curves is directly influenced by the amount of carotene fed and if the highest point on each curve is taken as the positive weight increase and divided by the quantity of carotene for that curve, the following results are obtained: 0.25 gm., 0.29 gm. and 0.22 gm. per γ carotene fed for the respective growth curves in figure 1. Thus within these limits growth is roughly, but not exactly, pro-

TABLE 1

Areas under the growth curves of rats receiving single feedings of carotene, when measured from the extreme ends of the average weight curves

AMOUNT OF CAROTENE FED	NUMBER OF ANIMALS USED	AREA IN SQUARE CENTIMETERS
28 γ	30	25
56 γ	21	50
112 γ	6	80

portional to the amount of carotene given in a single feeding; and in order to estimate as quantitatively as possible the vitamin A value of any substance by the single feeding method both the growth increase and the increase in time of survival should be taken into consideration. Measurement of the area under each curve apparently takes due account of both these factors.

In our first interpretation, such measurements were made using the negative control curve to bound one side of the area. A straight line drawn from the end of the carotene curve to the end of the negative control curve completed the area. Curves were plotted on cross section paper, of ⅒-inch squares, each representing 1 gm. on the ordinates and 1 day on the abscissa line. A planimeter was used for measuring the areas which are recorded in table 1.

The ultimate loss in weight by the experimental animals is likely to be a variable factor, particularly as it may be much accentuated in the last day or two before death, and variation in the length of the curve due to this may cause an undue variation in the area measured.

In our final interpretation it has seemed to us better to avoid the introduction of such errors by measuring the areas shown

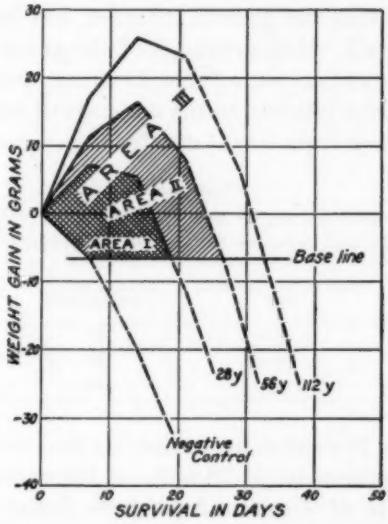

Fig. 1 Average gain curves showing how the areas were measured in securing the data shown in table 2. Shaded portion marked area I represents the area for the 28 γ carotene feeding, area II for 56 γ carotene, and area III for 112 γ carotene. The 'base line' is drawn horizontally through the point representing the average weight-loss of the negative controls at the end of the first week.

by the shaded portion in figure 1. A horizontal line is drawn through the point representing the end of the first week of the negative control curve, which includes records of all the negative control animals, and this is used as the base line for all areas. Areas as thus measured are given in table 2.

Within the limits of these experiments, therefore, the data of table 2 show that doubling the amount of carotene fed approximately doubles the area under the curve.

Cod liver oil diluted with vitamin A-free peanut oil was given in single feedings to litter mates of the rats of the carotene series described above. A fresh solution was made every 2 weeks. The average survival period for twenty-five rats receiving 7 mg. cod liver oil was 25.0 ± 0.6 days, while eighteen rats after receiving 14 mg. cod liver oil survived an average of 29.9 ± 1.1 days. The rats on 7 mg. cod liver oil survived the same length of time as those on 28 γ carotene, but figure 2 shows that the growth increase was less in the case of the cod liver oil. Measurements of the areas under the cod liver oil feeding curves were 6 and 12 sq.cm., respectively, and were thus in direct relation to the amounts of cod liver oil fed. The area obtained from the 14-mg. feeding was almost exactly

TABLE 2

Areas under the growth curves of rats receiving single feedings of carotene, when measured with reference to the base line explained in the text

AMOUNT OF CAROTENE FED	AREA IN SQUARE CENTIMETERS
28 γ	11
56 γ	25
112 γ	48

the same as for 28 γ carotene, indicating that 14 mg. of the cod liver oil were equivalent to 28 units of the carotene here used and to 28 units of vitamin A as here found by the single feeding method.

Litter mates fed daily amounts of cod liver oil for a period of 5 weeks gave the following data: eight rats receiving 1.0 mg. cod liver oil daily averaged 30.4 gm. of gain; two rats receiving 0.5 mg. cod liver oil daily averaged 19.5 gm. of gain. One unit of vitamin is seen to be contained in 0.5 mg. cod liver oil and therefore 14 mg. contain 28 units of vitamin A as found by the feeding method hitherto used. The findings by the single feeding method are thus in agreement with those of the method of continuous feedings through 4 weeks or more.

Kale purchased on the open market during March, April and May, and of the dark green curly leaved variety, was

given in the fresh state in single feedings to litter mates of the rats of the carotene series. Results are shown in figure 2; for eight rats receiving a single feeding each of 0.7 gm. kale, the average area under the curve was 65 sq.cm.; and for nine rats receiving 1.4 gm. kale, 136 sq.cm.

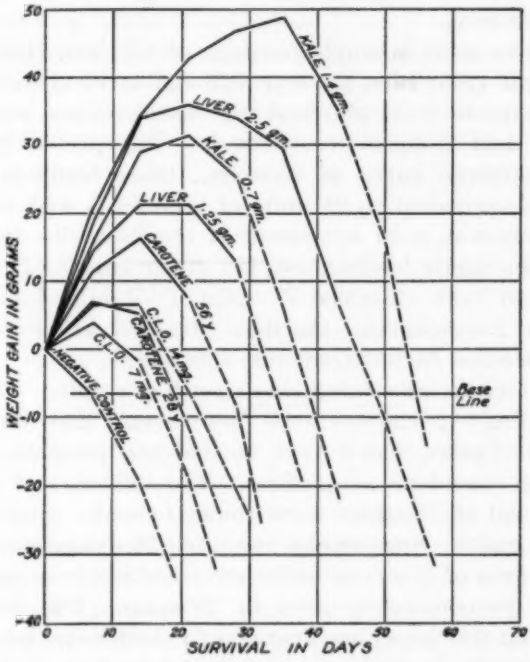

Fig. 2 Average gain curves for all animals on single feedings of different foods. (See also fig. 1.)

Using as a standard of reference the carotene curve of 25 sq.cm. which corresponds to 56 units, then 65 sq.cm. would be obtained by feeding 146 units of vitamin A, and 136 sq.cm. from feeding 305 units.

A group of rats received daily feeding of kale for 5 weeks and made the following average gains: six rats receiving 11 mg. daily of kale gained an average of 24 gm., and three rats receiving 5 mg. daily gained 12 gm. over that period.

The amount containing one unit of vitamin A is taken from this as 5 mg., therefore the single feedings of 0.7 gm. and 1.4 gm. by this method are seen to be equivalent to 140 and 280 units respectively. Thus the divergence from the older method shown by the new single feeding method was in this case 4 per cent for the lower level of feeding and 9 per cent for the higher level.

Calf liver used in single feedings of 1.25 gm. (three rats) and 2.5 gm. (four rats) gave growth and survival curves similar in shape to those obtained with carotene and areas were 41 sq.cm. and 83 sq.cm., respectively. Interpreted by means of the carotene curve of 25 sq.cm., these feedings of calf liver are equivalent to 91 units of vitamin A and 186 units. Thus there was good agreement of results at the two levels and by the single feeding test, the given sample of calf liver appears to have contained 73 units of vitamin A per gram.

Figure 2 summarizes the data obtained using the single feeding method for different test substances.

Some factors influencing single feeding results. Our carotene feeding experiments were fairly evenly distributed over a period of more than 1 year, so that any possible influence of season would be neutralized. The influence of sex was studied and the females were found to make smaller gains than the males; nine females receiving 56 γ carotene gave an average area of 17 sq.cm. under the growth curve, and twelve males a corresponding area of 28 sq.cm. The data used throughout this paper are averages for both sexes which were almost equally divided on each level of feeding.

Without attempting any general statement of the efficiency with which the animal organism is able to absorb and utilize large amounts of carotene, it may be pointed out that the responses of the experimental animals are similar (fig. 2) and approximate a quantitative relationship to the amount of material fed, whether this supplied the vitamin A itself or its precursor. Thus carotene represents the purified and concentrated form of a precursor of vitamin A. Cod liver oil contains vitamin A as such. Kale contains carotene in its

natural combined or admixed state. Calf liver owes its potency in part to vitamin A, some of which has been formed from the carotene of the green feed of the calf, some of which has been received directly from the milk of the calf's diet; while possibly there is also some unchanged carotene in the liver. Yet the method here developed appears applicable to all of these four types of material.

Such findings indicate that the single feeding method is practicable for determing the vitamin A values of widely varying types of food material if the substance to be evaluated is fed in parallel with carotene, or standardized cod liver oil, and if the sexes are evenly distributed on the different levels of feeding.

Applying the single feeding method in this way to kale shows it to have a vitamin A value of 200 units per gram of fresh moist kale. This value is comparable with that previously found for escarole, and considerably higher than any of the values which we have seen reported for spinach.

SUMMARY

Standarized rats depleted of their surplus body stores of vitamin A were given single feedings of carotene, of cod liver oil, of kale, or of calf liver. The weight curves throughout the period of survival after such feedings were charted on a fixed scale and the areas bounded by the resulting curves were measured with a planimeter. The lower boundary of such an area may be established either by drawing a line between the extreme ends of the average curves concerned or by the use of a horizontal base line through a point found from the performance of all of the negative controls as explained in the text. The latter mode of computation is here preferred.

Within limits amply sufficient for experimental work, increasing the amount of vitamin A or precursor given in a single feeding gave an approximately proportional increase in the area under the curve.

Carotene curves were used as a standard of reference and the curves obtained by feeding cod liver oil, kale, and calf

liver were interpreted quantitatively in terms of this standard. Direct comparison showed that the method of single feedings results in data which can be evaluated in terms of the same units and with findings in close agreement with those of the methods of feeding through periods of 4 or more weeks as hitherto regarded most accurate and conclusive. The same method can be used with a standardized sample of cod liver oil (or any other suitable material) as a basis of reference.

The area of the growth and survival curves for males was greater than for females. When both sexes are used they should be used in equal numbers (or other fixed proportion) throughout.

The single feeding method yielded an average finding of about 200 units of vitamin A value per gram of fresh moist kale, thus indicating kale to be among the richest of known natural sources.

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A VITAMIN B DEFICIENT RATION

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ONE CHART

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Various substances have been used as a source of vitamin G in vitamin B deficient rations. While autoclaved yeast or yeast concentrates have been most commonly employed, egg albumin (Chick and Roscoe, '29), special vitamin G concentrates (Guerrant and Dutcher, '32; and Roscoe, '30), and autoclaved meat (Sure, '33) have also been used. Milk is known to be relatively high in vitamin G and low in vitamin B. The recent work of Kuhn et al. ('33) and Booher ('33) indicates that highly potent vitamin G concentrates may be prepared from milk serum. In view of experiences at this laboratory (Bender, '32), as well as those reported from other sources, it seemed desirable to determine the merits of autoclaved dry milk and dry whey as sources of vitamin G for routine vitamin B assays. Although autoclaved dry whey has given uniform and consistently satisfactory results no detailed and comparative data have been reported. Such data are recorded in the present paper in comparison with the results obtained from autoclaved yeast and autoclaved milks.

EXPERIMENTAL

Dry whey, commercial dry skim milk as prepared by the Roller Process, and fluid skim milk were autoclaved for varying periods of time, with and without the addition of water as a moistening agent for the dry products. Sodium hydroxide solution, sodium bicarbonate, or hydrated lime were added to

the dry products prior to autoclaving to give a predetermined degree of alkalinity. The dry skim milk and dry whey samples were autoclaved at 120°C. for 1-, 2-, and 5-hour periods. Decomposition of the dry products as indicated by color and odor was minimized by moistening irrespective of the period of heating. The liquid skim milk sample autoclaved for 1 hour only was found to have a decreased pH and was adjusted to the pH of the original milk before drying. The 2-hour sample was autoclaved for 1 hour, its pH adjusted to that of the original milk, autoclaved for another hour and its pH again adjusted to that of the original milk. These two samples and a sample of the original milk were dried by the double roller process. Three samples of yeast were treated similar to the dry whey and dry skim milk, except that the period of heating for all such samples was 5 hours. One sample was a pure dry yeast, another was dehydrated yeast obtained from a different source, and the third was a sample of fresh baker's yeast. After autoclaving, all these products were air dried or oven dried at temperatures not exceeding 100°C. The pH value of the test substances was determined before and after autoclaving with but few exceptions. In those instances where sodium bicarbonate or hydrated lime was added before autoclaving sufficient amounts of these alkaline substances were used to bring the reaction to pH 7.5. The pH value of the original materials before the addition of alkaline substances and before autoclaving, varied between 5.8 and 6.4. After autoclaving, the pH of all samples had decreased to between 4.4 and 5.8 with the exception of two yeast samples which showed a pH value of 8.8. The reaction of the products before autoclaving did not appear to materially influence the amount of vitamin B remaining after the treatment.

The amount of residual vitamin B present in the autoclaved substances was determined by feeding 15 parts of the autoclaved material in place of the carbohydrate of the basal ration. The rate of growth and period of time before cessation of growth were used as the criteria for judging the

amount of vitamin B remaining in the product. The basal ration was as follows: vitamin free casein,[1] 20 parts; agar-agar, 2 parts; Steenbock salt mixture,[2] 4 parts; dextrin or sucrose, 62 parts; Crisco, 10 parts; and cod liver oil, 2 parts.

In order to determine whether an adequacy of vitamin G was carried by the autoclaved products, varying amounts of rice polish (for these particular determinations as high as 200 mg. per day) were fed as daily supplements following cessation of growth. It has previously been shown by Supplee, Kahlenberg and Flanigan ('31) that rice polish in this amount or less, supplies an adequacy of vitamin B for rapid growth when sufficient vitamin G is present. When good growth resulted after supplementation with the rice polish, it was concluded that the autoclaved product contained sufficient vitamin G.

The experiments were carried out with white rats which were housed in individual metal cages with screened bottoms (2 mesh to the inch). The animals selected for the experiments weighed 40 to 50 gm. and were 20 to 24 days old. With very few exceptions, an equal number of both sexes were used in each test group. The McCollum type feed dish was used.

The data summarized in table 1 are presented for comparative purposes to show the character of the results obtained from the different periods of heat treatment to which the various products had been subjected. The data show that the vitamin B of the autoclaved dry whey appears to be destroyed. That the dry whey still retains sufficient vitamin G for growth is illustrated by typical curves in chart 1. When vitamin B was supplied by 200 mg. rice polish fed daily in the absence of vitamin G, or when vitamin G was supplied by 15 per cent autoclaved dry whey in the absence of vitamin B growth did not result; however, when the two products were combined, excellent growth was obtained. These curves are typical of the results obtained. The results from the animals

[1] The vitamin free casein is distributed by the Casein Manufacturing Company of America, New York.

[2] Steenbock, H., and E. M. Nelson. J. Biol. Chem., 1923, vol. 56, p. 362.

TABLE 1

Effect on growth results of feeding with the basal ration 15 per cent autoclaved yeast, skim milk or whey products

GROUP	SAMPLE	AUTOCLAVE TREATMENT (120°C.)	NUMBER OF RATS STARTED	AVERAGE WEIGHT AT START	AVERAGE GRAMS GAIN PER WEEK FOR					
					First	Second	Third	Fourth	Fifth	Sixth
1	Yeast 2 [1]	Not autoclaved	4	46	29	31	22	14	14	6
2	Yeast 2	5 hours	32	46	23	15	10	6	3	2
3	Yeast 3 [2]	Not autoclaved	4	44	30	33	27	15	16	15
4	Yeast 3	5 hours	32	46	18	16	8	7	10	5
5	Yeast 4 [3]	5 hours	50	43	21	12	7	7	7	7
6	Dry skim milk	Not autoclaved	4	47	18	15	15	7	6	5
7	Dry skim milk	1 hour	16	45	9	10	6	3	1	2
8	Dry skim milk	2 hours	16	44	8	7	7	1	1	2
9	Dry skim milk	5 hours	16	45	8	6	5	−3	−1	0
10	Liquid skim milk	Not autoclaved but dried	4	41	17	13	14	5	3	4
11	Liquid skim milk	Autoclaved 1 hour and dried	8	42	12	12	3	−3	−7	0
12	Liquid skim milk	Autoclaved 2 hours and dried	4	41	12	9	5	−4	−6	−1
13	Dry whey	Not autoclaved	4	45	20	19	7	2	0	−1
14	Dry whey	5 hours	16	43	6	10	4	−3	−2	1
15	Dry whey	2 hours	60	45	13	14	−1 [4]			
16	Dry whey	2 hours [5]	8	45	10	4	−9	−9		

[1] Yeast 2—pure dry yeast.
[2] Yeast 3—dehydrated yeast.
[3] Yeast 4—fresh baker's yeast.
[4] Animals used for other tests as soon as weight remained constant or decreased.
[5] Sucrose replaces dextrin.

receiving the autoclaved skim milks were quite similar to those obtained from dry whey. When dextrin is used as the carbohydrate in the basal ration, the results are about the same as those obtained when sucrose is used, although the average length of life on the vitamin B deficient dextrin ration is a few days longer, and somewhat more variable.

The explanation of the observed difference in the growth reaction of the animals receiving autoclaved yeast and those receiving the autoclaved milk products must remain a matter of speculation. The results from the autoclaved yeast are in

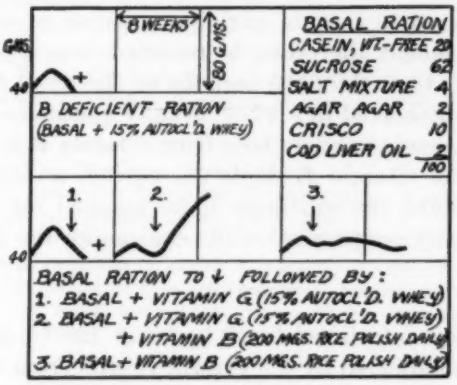

Chart 1 Growth response of rats receiving the basal ration with and without vitamin B and G supplements.

harmony with those reported by Sure ('33). However, other investigators, such as Chase and Sherman ('31), Evans and Lepkovsky ('31) and Kemmerer and Steenbock ('33) report results and growth reactions from autoclaved yeast similar to those reported herein from the autoclaved milk products. The consistency and satisfaction of the results from 15 per cent dry whey autoclaved for 2 hours as a source of vitamin G free from vitamin B have been such as to warrant its extended use for vitamin B assays made at this laboratory. The following is an outline of the procedure which has been successfully used for some time past.

Dry whey[3] is moistened with water to a paste of medium consistency and autoclaved for 2 hours at 120°C. in shallow containers, the paste being about 2 inches in depth. As soon as it is taken from the autoclave it may be intimately mixed with dextrin, and air or oven dried; or it may be spread in thin layers and dried at 60° to 100°C. and subsequently ground. The dry material is incorporated in the basal ration previously described to the extent of 15 per cent, replacing an equivalent amount of dextrin or sucrose. Some samples of the particular dry whey under consideration have been quite free from vitamin B when received, while other samples have been found to contain variable amounts of this factor. Autoclaving should, therefore, be resorted to as a precautionary measure. Growth of the animals on the basal ration containing the autoclaved dry whey usually continues for about 21 days, if a casein entirely free from vitamin B is used. At the end of the vitamin B depletion period, as indicated by decline in weight, the substance to be assayed for vitamin B is fed as a daily supplement while continuing the B deficient ration.

SUMMARY

1. Dry whey autoclaved for 2 hours at 120°C. contains no vitamin B as indicated by the growth results from white rats.

2. Fifteen per cent of the autoclaved dry whey used in these experiments supplies an amount of vitamin G sufficient for good growth.

3. More consistent and satisfactory results have been obtained at this laboratory from autoclaved dry whey, as a source of vitamin G free from vitamin B, than from autoclaved yeast.

[3] The dry whey used for these experiments is known commercially as Peeble's Lacto-Milk and is distributed by the Western Condensing Co., San Francisco, California.

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THE VITAMIN B SUPPLEMENTATION OF MILK

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ONE CHART

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The primary results, and in many instances the deductions from published data, clearly show that vitamin B directly affects growth and efficiency of food utilization (Graham and Griffith, '33; Sure, '32; Sherman, '31). An analysis of certain data (Coward et al., '33; Graham and Griffith, '33) seems to support the view that there is a variable complementing effect between dietary constituents, vitamin in character or otherwise, and vitamin B which may account for variations in growth rate. Whether the observed growth is always due to the particular vitamin specified, or whether it is due to particular balanced relationships between a plurality of vitamins may be a matter of conjecture in many instances. Notwithstanding the lack of suitable methods necessary to determine the specific mode of action of vitamins B and G, an accelerated rate of growth of white rats has been obtained at this laboratory for many years from milk and its derivatives supplemented by substances known to contain relatively large amounts of vitamin B. The data reported herein are concerned with variations in the growth rate as affected by vitamin B with and without accompanying milk solids.

EXPERIMENTAL

It has been shown previously (Supplee et al., '31) that crude rice polish and a water soluble vitamin concentrate prepared

from milk, when supplementing a suitable basal ration, permitted excellent growth; in one instance a gain of 59 gm. per week was noted. Seven per cent of rice polish and an equal amount of the milk vitamin concentrate have supported, up to the present time, normal growth, reproduction and lactation in white rats through eight successive generations. Such animals as it has been practicable to retain for the purpose of determining longevity on this diet have shown an average age at the time of death of 114 (maximum 152) weeks for the females and 88 (maximum 133) weeks for the males. While this ration has not been assayed to determine its relative vitamin B and G content in terms of empirical units, it is known to contain relatively high proportions of each.

In view of these and other data which have been obtained from milk and rice polish or their derivatives, the test substances required for the following studies were confined to these two products. The plan of procedure was to determine the relative vitamin B content of rice polish derivatives and milk solids according to the Chase and Sherman ('31) assay procedure. The assayed substances were then fed in varying amounts, singly and in combination, for the purpose of ascertaining the growth response from different amounts or 'units' of the vitamin; the same technic as used for the assays was followed.

The basal ration free from vitamin B and G consisted of vitamin-free casein,[1] 20 parts; powdered agar agar, 2 parts; Steenbock ('23) salt mixture, 4 parts; dextrin, 63 parts; Crisco, 10 parts; and cod liver oil, 2 parts. This ration was converted to a vitamin B free ration containing an adequacy of vitamin G by replacing 15 parts of dextrin with an equal

[1] The casein used in this ration is obtained free from the water soluble vitamins during direct preparation from fluid milk by controlling the ionic condition of the aqueous suspension. It is not subjected at any time during the course of preparation to the solvents usually used in the treatment of commercial grades of casein for the purpose of rendering them vitamin-free. This product has been commercially available for about 2 years and is distributed by the Casein Manufacturing Company of America, New York.

amount of autoclaved dry whey.[2] The depletion period on this ration is about 3 weeks, at which time weight of the rats is from 60 to 80 gm. If it is desired to convert the basal ration to one free, or substantially free, from vitamin G, but with sufficient vitamin B for normal growth, 2 per cent crude rice polish is added.

Aqueous extracts were prepared from crude rice polish by violently agitating 1 part of the polish with 4 parts of water at 7°C. for 4 hours. The insoluble matter was removed by mechanical means leaving a solution containing from 4 to 5 per cent dry matter. Assays of this material showed that about 25 mg. of dry substance per day were required to promote a 3 gm. gain per week for a period of 6 weeks.[3] A more highly purified concentrate[4] prepared from rice polish according to the method described by Sure ('32) required 2.8 mg. of dry substance for the same growth response. Assays of the dry milk solids[5] showed that 270 mg. were required for the 3 gm. gain in weight.

In order to eliminate certain influences which might effect the interpretation of the results obtained from feeding mixtures of the vitamin B concentrates and milk solids, it was desirable to determine whether the freshly prepared aqueous extract underwent deterioration in potency during the heat treatment to which milk is subjected in the atmospheric double roller process of desiccation. Supersaturated solutions of pure lactose varying from 27 to 40 per cent concentration were made with the freshly prepared water extract of

[2] It has been found that one particular brand of dry whey frequently contains little or no vitamin B. Complete absence of this vitamin in different shipments cannot be relied upon. Therefore it is our practice to autoclave the moistened product for 2 hours at 120°C. This product is known as Peeble's Lacto Milk and is distributed by the Western Condensing Co., San Francisco, California.

[3] The crude rice polish from which these extracts were prepared required 100 mg. for 1 unit. Other samples of crude rice polish have been more potent in vitamin B, requiring only 60 to 75 mg. per unit.

[4] We are indebted to Dr. L. W. Bass, of the Borden Research Department, for furnishing a sample of this concentrate prepared by Doctor Sure.

[5] The dry milk used throughout the experiments recorded in this paper is known as Dryco Brand. It is irradiated by ultraviolet rays prior to desiccation by the Just Process.

rice polish. These solutions were converted to dryness by proper manipulation of the drying apparatus and yielded the beta type lactose impregnated with known amounts of the rice polish extract solids. The temperature to which the mixture was subjected varied progressively from about 110°C. to 135°C. during a period of about 9 seconds. The vitamin B assays of various samples of the heat treated product showed that substantially 28 mg. of the extract solids were required for 1 rat unit, as compared with 25 mg. required prior to heating. The daily amount of this vitamin B fortified lactose necessary to carry the extract solids for 1 unit varied from 300 to 500 mg., depending upon the initial concentration. On the basis of these results as well as other data, it is concluded that the destruction of vitamin B during the heat treatment was relatively slight or at least not greater than 10 per cent.

The assay results from the different test substances were used as a guide for determining an appropriate range of graduated doses of vitamin B which it was desired to feed singly or in combination. Since it was desired to maintain the amount of growth during the test period within a range which would not be in the least affected by maturity size, fractions of the amount required for the 3 gm. unit gain or small multiples thereof were used. The test substances were supplied as daily supplements following the depletion period. All experimental conditions were maintained the same as prevailed during the determination of the assay results. Where dry milk solids were used singly or in combination with rice polish extracts, the test substances were subjected to the heat treatment prevailing in the desiccation of milk by the roller process. Where rice polish concentrates were fed alone, desiccation of the fluid extracts was accomplished by drying with the basal ration at a temperature not exceeding 80°C. or by concentrating in vacuum and subsequently drying by the spray method commonly employed in the drying of milk. The only exception to this procedure was in the case where the aqueous extracts were dried with the lactose solutions in the manner

mentioned above. Preliminary tests showed no significant difference in potency resulting from these methods of desiccation. The averages of the results obtained are recorded in table 1 and significant relationships are shown graphically in chart 1. In this chart the expected normal rate of growth is plotted (dotted line) on the assumption that each rat unit

TABLE 1

Growth response due to vitamin B

MATERIAL FED AS DAILY SUPPLEMENT	SUPPLEMENTING MATERIAL FED PER DAY			NUMBER OF RAT UNITS OF VITAMIN B[1]	AVERAGE GAIN PER WEEK	FOOD INTAKE PER GRAM GAIN
	Total solids	Milk solids	Rice polish extract solids			
	mg.	*mg.*	*mg.*		*gm.*	*gm.*
Water extract of rice polish	9.4		9.4	0.38	0.4	46.3
Water extract of rice polish	11.7		11.7	0.47	0.6	17.8
Water extract of rice polish	13.9		13.9	0.56	2.4	9.0
Water extract of rice polish	18.8		18.8	0.75	1.6	9.8
Water extract of rice polish	23.4		23.4	0.94	4.2	5.9
Water extract of rice polish	27.7		27.7	1.11	5.3	4.7
Water extract of rice polish	37.7		37.7	1.51	6.3	3.3
Dry milk solids	200.0	200		0.70	2.5	11.0
Dry milk solids	400.0	400		1.50	3.9	7.9
Dry milk solids	800.0	800		3.00	9.9	4.2
Water extract of rice polish + dry milk	100.0	95	5.0	0.55	1.1	20.8
Water extract of rice polish + dry milk	200.0	190	10.0	1.10	4.5	6.5
Water extract of rice polish + dry milk	400.0	380	20.0	2.20	8.8	4.1
Vitamin B concentrate (Sure)	0.5		0.5	0.18	Died	....
Vitamin B concentrate (Sure)	1.0		1.0	0.36	1.2	18.3
Vitamin B concentrate (Sure)	2.0		2.0	0.72	1.1	23.2
Vitamin B concentrate (Sure)	3.0		3.0	1.07	4.3	6.2
Vitamin B concentrate (Sure) + dry milk	200.5	200	0.5	0.91	3.6	7.2
Vitamin B concentrate (Sure) + dry milk	201.0	200	1.0	1.08	6.5	4.7
Vitamin B concentrate (Sure) + dry milk	202.0	200	2.0	1.42	8.7	3.8
Vitamin B fortified lactose	279.0		20.0	0.71	2.5	9.9
Vitamin B fortified lactose	360.0		26.0	0.93	3.8	6.4
Vitamin B fortified lactose	558.0		40.0	1.39	7.5	4.4
Vitamin B fortified lactose	720.0		52.0	1.86	8.6	3.9

[1] Calculated on basis of amount necessary for 1 rat unit: 25 mg. rice polish extract solids. 28 mg. rice polish extract solids (fortified lactose). 2.8 mg. Sure's vitamin B concentrate. 270 mg. milk solids.

produces a 3 gm. gain per week. It is a reference line which is included for comparative purposes only. The slope of the other lines was calculated from the various data according to the general formula ($y = mx - n$).

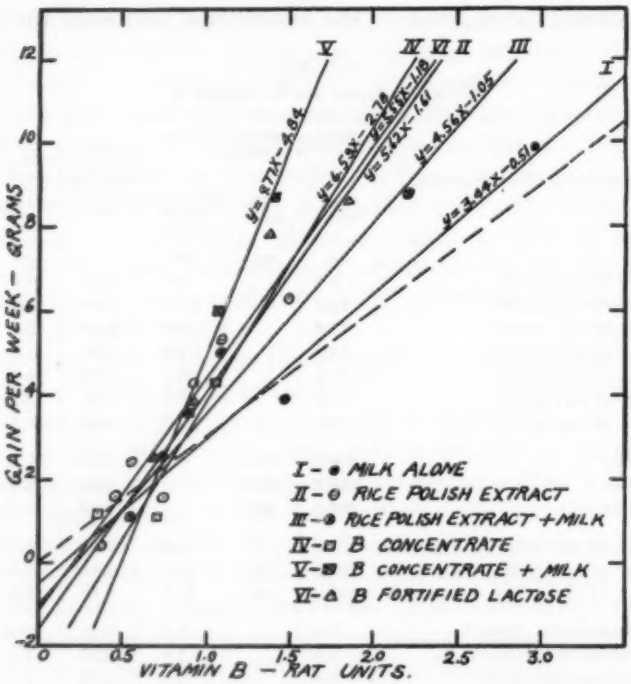

Chart 1 Relation of vitamin B to growth response of various rice polish substances and milk, alone and combined.

The tabular data, and especially the graphs show a proportional relationship between the rate of growth and the vitamin B intake. In all instances, however, with the possible exception of the results obtained from milk alone, the growth rate is greater than that which was to be expected by calculation. The results from the aqueous extracts of rice polish (curves II and VI) and the rice polish concentrate

prepared according to the method of Sure (curve IV) show growth promoting properties of the same order of magnitude when the 3 gm. unit gain is the basis of appraisal of their vitamin B content. However, it cannot be assumed that the similarity of results from these three substances is sufficient to warrant the firm conclusion that the observed rate of growth represents a specific and inflexible criterion which may be expected from like amounts of vitamin B accompanying substances of heterogeneous composition.

The different rates of growth obtained from these vitamin B concentrates when fed with milk are interpreted as evidence of a variable growth promoting effect resulting from different proportions of vitamin B and other constituents of the diet, vitamin in character or otherwise. This observation seems to be supported by the recent work of Coward ('33) and is in conformity with previous results obtained at this laboratory (Supplee, '31). The data show that the lowest growth rate per unit of vitamin B was obtained from the unsupplemented milk (curve I); the highest rate was from the milk supplemented by the highly purified Sure concentrate (curve V); an intermediate rate of growth was obtained from the milk supplemented by the less potent aqueous extract of rice polish. While the growth rate resulting from this last test mixture was substantially greater than from milk alone, it was lower than that obtained without the accompanying milk solids.

While a substantial degree of tolerance must be allowed for the mathematical presentation of biological data of this character, the results seem to leave no reasonable doubt that definite differences in growth promoting properties are exhibited by the supplemented and unsupplemented products. Since the unit amount of vitamin B was determined by unit gain in weight, it is obvious that if different substances or variations in the optimal balance between substances affect the rate of growth, a false criterion for vitamin B will result. For example, the rate of growth shown by the unsupplemented aqueous extract of rice polish may have been due to not only

its vitamin B content but to other accompanying substances as well. However, the experimental plan and method of expressing the data called for uniformity in assuming that growth response resulting from the assay procedure was entirely attributable to vitamin B. When this product supplemented milk, only its actual vitamin B content would be manifested; this was apparently lower than was seemingly revealed when it was fed alone. The results from the more highly purified Sure concentrate which presumably contained less growth promoting substances other than vitamin B, seem to be in harmony with this explanation.

The data as a whole show that rate of growth is increased and greater efficiency of food utilization results from increasing amounts of vitamin B provided in the ration. These manifestations bear a direct linear relationship to the amount of vitamin B present, irrespective of supplementing milk solids. The results from extended ad libitum feeding experiments with dry milk supplemented with crude rice polish or its aqueous extracts support the data reported herein, in showing substantially the same linear relationship between the amount of vitamin B supplied and the growth rate. When 10 per cent unsupplemented dry milk solids were used in the ration the average gain was 8.9 gm. per week; when a further supplementation of 2, 3 or 4 per cent of rice polish or its equivalent aqueous extract was provided, the average gain per week was 13.6, 15.5 and 18.7 gm., respectively. While the various data show a direct relationship between growth rate and the amount of vitamin B supplied, a specified rate of growth cannot be accepted as an infallible criterion for judging the absolute amount of vitamin B present.

Although milk is not generally considered to be a rich source of vitamin B from a comparative standpoint, human experience has shown no general evidence of an acute deficiency of this factor in the normal milk fed infant. The data presented herein would seem to show that the amount of vitamin B required to maintain a given rate of growth may be variable depending upon the presence of other dietary sub-

stances. The results indicate that substances which have a sparing effect upon the vitamin B demand are contained in milk, or else, the particular balanced relationship between the vitamin B and other factors contained in milk are such that the nutritive character of milk as a whole cannot be considered as particularly deficient in this vitamin. Nevertheless, various observers (Hoobler, '28; Dennett, '29; and Barry, '30) have noted that the plane of nutrition of infants and children may be improved, in so far as gain in weight may be accepted as a valid criterion, by supplementing the milk dietary with vitamin B. An extensive clinical study of the merits of vitamin B fortified dry milk similar to that used in these experiments has been recently reported by Gaynor and Dennett ('34), wherein it is stated that: "This increase in weight (referring to the gain made by infants receiving the vitamin B fortified milk) is not due to an increased caloric intake as demonstrated by the fact that the average daily intake per pound body weight in the vitamin B group is 35 as compared with from 45 to 55 in other groups."[6] This observation is of particular interest in view of the data recorded in this paper wherein the growth promoting properties of the same product were subjected to an integrated study with experimental animals.

CONCLUSIONS

1. Potent vitamin B extracts may be prepared from rice polish by simple extraction procedures; such concentrates may be condensed in vacuum, spray dried, or dried by the Just Process without significant diminution in potency.

2. There is a direct linear relationship between the rate of growth of white rats and the amount of vitamin B supplied in the ration. However, a specified rate of growth cannot be accepted as an infallible criterion for judging the absolute amount of vitamin B present; the presence of other dietary constituents or definite relationships between such constitu-

[6] We are indebted to Doctors Gaynor and Dennett for the privilege of reading their manuscript now in press.

ents and the amount of vitamin B seem to determine the rate of growth observed.

3. Dry milk containing a concentrated water extract of vitamin B obtained from rice polish showed greater growth promoting properties than was shown by the milk not containing such supplement.

4. Vitamin B, either with or without milk supplementation, increases the efficiency of food utilization as judged by the growth response per unit of food intake.

5. A highly purified vitamin B concentrate prepared from rice polish gave results comparable to those obtained by simple water extraction; however, the former was approximately ten times more potent than the latter as judged by its effectiveness when used with milk or when assayed without the milk supplement.

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